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XLI. THE DISTRIBUTION OF ESTERASES IN THE ANIMAL BODY.

By AGNES ELLEN PORTER, *Carnegie Research Fellow.*

From the Royal College of Physicians Laboratory, Edinburgh.

(*Received October 7th, 1916.*)

It is to the organs more directly concerned with digestion that one naturally looks for fat-splitting ferments. Yet, as a matter of fact, the body appears to be permeated with ferments which are entrusted with the delicate mechanism of equilibrium for which their peculiar reversible action is fitted, and upon which nutrition, metabolism, and life itself depend. It may be boldly presumed that wherever any constituent of an organ is found in a constant proportion, a ferment will be found preserving that proportion. A well-known illustration of this relation may be seen in the action of the glycogen-sugar ferment of the liver and blood. Going upon this assumption, one would expect to find lipase in organs containing glycerol esters, lecithase in organs containing lecithin, wax-splitting ferments in organs containing higher alcohol esters. On the other hand, it does not follow that the presence of ferments indicates also the presence of their substrates; the ferment may be elaborated, as in the case of pancreatic steapsin, for use in another sphere of action than the site of its origin.

Fat-splitting ferments have been described in many different tissues by a number of authors, among others Loewenhart [1902] and Rona [1911, 1, 2]. Other esterases such as cholesteryl esterases and wax-splitting ferments, that is ferments which are able to split the esters of higher alcohols, also occur, as I have recorded in a former paper.

A parallel has been drawn between lypolytic power and resistance to tuberculosis. Winternitz and Meloy [1910] who have described lipase (decomposing ethyl butyrate) in a number of human organs, find a diminution

of this ferment in the kidneys, liver, lungs and blood in tuberculosis. Achard and Clerc [1902, 1904] have also noticed a diminution in Hanriot's serum lipase in tuberculous conditions which were not improving.

In order to investigate the distribution of different esterases in the animal body, and incidentally to compare lipolytic activity with the known divergence as regards resistance to tuberculosis of certain species and organs, extracts were made of the following organs and examined:

	Human	Ox	Sheep	Pig	Cat	Rabbit	Guineapig
Pancreas	+	+	+	+			
Liver	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+
Kidney	+	+	+	+	+	+	+
Brain	+	+	+	+			
Suprarenals	+	+	+	+			
Thyroid	+	+	+	+			
Thymus		+	+	+			
Lymphatic glands	+	+	+	+			
Haemolymph glands		+					
Pituitaries		+	+				
Bonemarrow		+					
Salivary glands				+			
Skin	+						

In some cases, especially with human organs, which were more or less pathological, and usually less active than the normal organs obtained from the other species, material from more than one individual was examined. In the case of human lymphatic glands seven samples of tuberculous glands in addition to non-tuberculous were under investigation.

The best medium known for extracting lipase from tissues is glycerol. Lipase is soluble in pure glycerol, as also are salts. Fatty acids, alcohols and esters are only very slightly soluble, but enough to aid lipolysis and also the titration of these usually difficult soluble substances. Glycerol also preserves the lipase by abstracting water, which injures the ferment. Glycerol is indeed well known as a preservative and anti-putrefactive, being used in commerce, for example in rennet preparations, etc., for that purpose. Although ferment solutions extracted from organs are usually difficult to preserve from putrefaction, the glycerol extracts made from organs in this investigation all became sterile within a few days. That this did not depend on the direct action of the glycerol on the organisms, but rather on its preservative influence on bacteriolytic substances present, was proved by the varying rate at which the organs became sterile. Liver became sterile at

once; lung was perhaps the slowest of all. This sterility allowed of certainty in ascribing lipolytic action to the organ from which the extract was made.

The method of extraction adopted was that recommended by Kanitz [1905], and is very simple. Glands were minced down, and pure glycerol added, in the proportion of one-third gland and two-thirds glycerol. After two days in contact, the extract was filtered through gauze, as lipases are easily injured by filtration. A small amount of fat may come through mechanically, but this is of no consequence if the autolytic activity of the extract itself is always measured in the presence of activating calcium chloride.

The following esters were used:

(1) Glycerol esters: butyrin, tripalmitin, tristearin, olive oil, castor oil, cod liver oil, and lecithin.

(2) Cholesterol esters: lanolin, cholesteryl propionate, cholesteryl palmitate, cholesteryl stearate (made according to Mair's [1914] directions).

(3) Beeswax, containing myricyl palmitate.

(4) Spermaceti, containing cetyl palmitate.

(5) Chinese wax, containing ceryl cerotate.

(6) Carnauba wax, containing myricyl cerotate.

The glycerol esters, with the exception of butyrin, were made up in 20 % solutions or emulsions. Butyrin was used undiluted, water being added in proportionate amount. Olive, castor, and cod liver oil were neutralised and diluted to approximately 20 %, 10 % of alcohol being present. Ovolecithin, obtained from Merck, was dissolved to 20 % in cold alcohol. Its acidity was deducted. Tristearin, tripalmitin, and the higher alcohol esters were dissolved in 40 % benzene, or xylene, and were warmed before use to allow of measurement, these substances being very insoluble. They were either neutralised in boiling methyl alcohol with methyl alcoholic potash, the alcohol being removed by evaporation, or, more often, as this first method appeared somewhat to increase insolubility, they were dissolved and used in an acid state, the initial acidity being deducted. It should be mentioned that when the fats were first obtained they were boiled in order to destroy any ferment present, as commercial preparations of fats are not always free from traces of lipases. Lecithin, although boiled, decomposes slowly in the presence of water. This process is, however, much hastened by the presence of gland extract, which contains lecithase.

The gland extracts added to fat alone, are quite inactive and require an activating agent. Activation is believed to depend upon a removal of

the inhibitory products of ferment action. Thus $CaCl_2$, recommended by Kanitz [1905], has been shewn by Pekelharing [1912] to act by forming insoluble soaps of calcium, and so by precipitation, to remove the products which are hindering further action. The action of bile salts is quite opposite to this, by promoting solubility, especially in the duodenum, they promote the removal of the end products of lipolysis by their absorption through the membrane.

In testing for esterases, in each tube were placed 1 cc. of ester solution, 1 cc. of glycerol gland extract, 0·5 cc. of 5 % $CaCl_2$, and 0·5 cc. of alcohol. Controls were made containing 1 cc. of gland extract, with a similar amount of benzene, alcohol, and $CaCl_2$, but no ester. Esters were also tested alone with $CaCl_2$ and alcohol, in order to deduct their end acidity from the total acidity found after lipolysis. The mixtures were kept at room temperature and were neutralised with N/10 NaOH repeatedly during 24 hours. It is difficult to set a time limit, as, especially in the case of glycerides emulsified in water, neutralisation often restarts ferment action which had ceased, yet some mixtures act as quickly as they can be neutralised, while others may be exceedingly slow, but are never quite finished. This was especially the case with butyrin. Most mixtures could not be carried beyond a certain definite limit, which was quickly reached. This was especially the case with the higher alcohol esters and was no doubt largely due to the fact that in benzene they were mainly in a dissolved condition, which certainly aids hydrolysis.

Results.

In the different glands examined fat- and wax-splitting ferments were found, which appeared to be quite independent of each other. The gland extracts differed greatly in lipolytic properties, both in strength and kind. Butyrinase was always present, although varying greatly in strength. As lecithin is so essential a constituent of cells, it is not surprising that lecithinase should appear quite as constantly. Lecithin is, however, decomposed by water and $CaCl_2$; the presence of lecithase is only determined by increased rate of decomposition so that the demonstration of small quantities of the ferment is not very satisfactory. Action on unsaturated glycerides such as olein was also rather commonly found, but often very slight. Many organs had no effect on tristearin; those which had the most influence on this rather insoluble fat were also able to deal with the higher alcohol esters, suggesting,

once again, a connection between solubility and lipolytic power. Ferments hydrolysing the esters of the higher alcohols were found in one sample of human skin in quite remarkable strength. In pancreas extract they were also strong, and were present in a lesser degree in the liver, thymus, suprarenal, lymphatic, and other glands. On comparing the lipolytic power of the different species examined, some interesting facts are apparent. The pig has been regarded as possessing more lipolytic power than the other species, but this superiority is practically entirely limited to the pancreas. Lipases are widely distributed in the organs of the pig, but in no greater measure than in the organs of other animals. Certain organs of the sheep, especially pancreas, liver and spleen, are considerably less lipolytic than in the pig, so that these organs certainly afford no explanation of the greater susceptibility of the pig to tuberculosis. If, however, the lymphatic glands of these two species are compared, a very different relationship will be found. While sheeps' lymphatic glands were found remarkably active, being the most active lymphatic gland extracts examined, pigs' glands were decidedly the weakest. The pig is susceptible to glandular tuberculosis. As ingested tubercle is believed to enter the body by filtration through lymphoid tissue (tonsils and ileo-caecal region), and then via the lymphatic glands, this lipolytic activity in the sheeps' lymphatic glands may well explain the resistance of the sheep to this disease. With the exception of one sample of human skin, human organ extracts were found disappointingly weak on the whole. On account of their source, and more or less pathological condition, they cannot be fairly compared with normal organs from other species. Nevertheless, although the results given by them are not striking, they indicate a widespread, decided and fairly even lipolytic activity over the very different varieties of fats used. Except for lecithase in the brain, the only superiority which human glands displayed was seen in a kidney shewing fatty degeneration, where there was unusual lipolytic activity, especially in the form of butyrinase. The guineapig, which is so susceptible to tuberculosis, proved decidedly weak in lipolytic power, except with regard to butyrin. The cat, on the other hand, resistant to tuberculosis, yielded stronger ferments, more especially against the higher alcohol esters. It is interesting that the brain, which contains cholesterol, but no cholesteryl ester [Lorrain Smith and Mair, 1912], possesses no cholesteryl esterases. On the other hand, those organs which are known to contain cholesteryl esters, *i.e.*, skin and suprarenals, contain also higher alcohol esterases.

In the following tables, shewing esterase activity, any primary fat acidity has been deducted, but not gland acidity. The acidity of the gland alone is, however, always given below. The gland acidity, and low or negative esterase figures close to it, are printed in italics, so that positive results are more conspicuous. The numbers throughout represent cc. of N/10 NaOH required to neutralise the mixtures within 24 hours.

Esterase Activity.

Pancreas.

	Substrate	Maximum acidity*	Human	Ox	Sheep	Pig
1.	Butyrin	19·9	11·7	16·0	16·0	16·0
2.	Palmitin	7·2	*0·6*	1·0	—	5·6
3.	Stearin	6·7	*0·4*	0·7	0·75	5·6
4.	Cod-liver oil	6·7	3·6	1·9	1·75	5·0
5.	Castor oil	6·6	3·0	2·0	2·0	4·4
6.	Olive oil	7·0	4·4	2·7	2·0	5·0
7.	Lecithin	—	—	7·0	7·0	7·0
8.	Lanolin	7·0	7·0	1·0	1·6	5·1
9.	Beeswax	6·7	*0·4*	0·9	1·1	5·1
10.	Cholesteryl stearate ...	6·2	*0·7*	1·0	1·0	3·0
11.	Cholesteryl palmitate ...	6·4	*0·3*	1·0	1·0	3·5
12.	Cholesteryl propionate ...	9·0	*0·4*	1·6	1·2	5·0
13.	Carnauba wax	6·6–6·8	*0·4*	0·6	*0·6*	3·2
14.	Chinese wax	—	—	0·6	*0·6*	3·3
15.	Spermaceti	8·8–9·6	—	0·8	1·25	4·5
	Gland control	—	*0·3*	*0·2*	*0·35*	*0·9*

* The maximum acidity is calculated from the saponification value.

Lung.

	Substrate	Human	Ox	Sheep	Pig I	Pig II	Cat	Rabbit	Guinea-pig
1.	Butyrin	1·0	0·6	1·8	—	0·4	2·2	*0·5*	1·2
2.	Palmitin	*0·3*	*0·15*	*0·1*	—	*0·1*	*0·35*	*0·3*	*0·2*
3.	Stearin	*0·3*	*0·15*	*0·35*	—	*0·1*	*0·35*	*0·3*	*0·2*
4.	Cod-liver oil ...	*0·3*	*0·1*	*0·25*	—	*0·2*	*0·35*	*0·3*	*0·2*
5.	Castor oil	*0·3*	*0·15*	*0·3*	—	*0·2*	*0·35*	*0·2*	*0·2*
6.	Olive oil	*0·4*	*0·2*	0·6	0·8	*0·2*	0·45	*0·3*	*0·2*
7.	Lecithin	*0·7*	1·8	1·9	1·4	1·5	1·5	*0·6*	*0·7*
8.	Lanolin	*0·5*	*0·15*	*0·3*	—	*0·2*	*0·25*	*0·2*	*0·15*
9.	Beeswax	*0·3*	*0·15*	*0·45*	—	*0·2*	*0·2*	*0·3*	*0·2*
10.	Cholesteryl stearate	*0·4*	—	—	—	—	*0·2*	*0·3*	*0·2*
11.	Cholesteryl palmitate	*0·4*	—	—	—	—	*0·3*	*0·3*	*0·2*
12.	Cholesteryl propionate	*0·5*	—	—	—	—	*0·3*	*0·3*	*0·2*
13.	Carnauba wax ...	*0·3*	—	—	—	—	*0·2*	—	—
14.	Chinese wax ...	*0·3*	—	—	—	—	*0·25*	—	—
15.	Spermaceti	*0·6*	—	—	—	—	*0·4*	—	—
	Gland control ...	*0·4*	*0·15*	*0·3*	*0·2*	*0·2*	*0·25*	*0·4*	*0·2*

Lymphatic glands.

Substrate	Human Tub.	Human Normal	Ox	Sheep	Pig
1. Butyrin	0·6	0·7	2·2	12·5	1·0
2. Palmitin	*0·4*	*0·15*	1·0	1·0	*0·2*
3. Stearin	*0·3*	*0·15*	1·0	1·0	*0·25*
4. Cod-liver oil	*0·25*	*0·15*	1·1	3·5	0·5
5. Castor oil	*0·3*	*0·15*	1·1	3·7	*0·3*
6. Olive oil	*0·35*	*0·15*	1·1	3·5	0·5
7. Lecithin	1·4	0·8	2·25	2·5	1·0
8. Lanolin	*0·35*	*0·15*	0·9	0·8	*0·25*
9. Beeswax	*0·35*	*0·15*	1·2	1·4	*0·25*
10. Cholesteryl stearate ...	*0·25*	*0·15*	1·0	1·0	*0·35*
11. Cholesteryl palmitate ...	*0·4*	*0·15*	1·0	1·0	*0·3*
12. Cholesteryl propionate ...	*0·4*	*0·15*	0·8	*0·7*	*0·3*
13. Carnauba wax	*0·35*	*0·15*	—	—	*0·2*
14. Chinese wax	*0·35*	*0·15*	—	—	*0·25*
15. Spermaceti	*0·45*	*0·25*	—	—	*0·4*
Gland control	*0·25*	*0·15*	*0·4*	*0·5*	*0·2*

Brain.

Substrate	Human	Ox	Sheep	Pig
1. Butyrin	1·2	0·55	0·7	0·7
2. Palmitin	*0·2*	*0·2*	*0·4*	*0·2*
3. Stearin	*0·2*	*0·2*	*0·4*	*0·2*
4. Cod-liver oil	*0·3*	*0·2*	*0·4*	*0·3*
5. Castor oil	*0·3*	*0·25*	*0·4*	*0·3*
6. Olive oil	*0·3*	*0·3*	*0·4*	*0·3*
7. Lecithin	7·0	2·3	2·3	2·0
8. Lanolin	*0·2*	*0·2*	*0·4*	*0·15*
9. Beeswax	*0·25*	*0·2*	*0·5*	*0·2*
10. Cholesteryl stearate ...	*0·2*	*0·2*	0·9	*0·2*
11. Cholesteryl palmitate	*0·2*	*0·2*	*0·4*	*0·2*
12. Cholesteryl propionate	*0·3*	*0·2*	*0·4*	*0·2*
13. Carnauba wax ...	*0·2*	—	—	—
14. Chinese wax	*0·2*	—	—	—
15. Spermaceti	*0·3*	—	—	—
Gland control ...	*0·2*	*0·2*	*0·4*	*0·2*

Haemolymph glands.

Ox
1·2
0·5
0·5
0·45
0·45
0·6
1·3
0·5
0·5
0·5
0·4
0·4
0·8
0·5
0·5
0·35

Suprarenals.

Substrate	Human	Ox	Sheep	Pig I	Pig II
1. Butyrin	*0·65*	1·7	1·9	1·25	1·4
2. Palmitin	*0·65*	1·1	*0·65*	*0·6*	0·75
3. Stearin	*0·65*	1·1	*0·6*	*0·6*	*0·65*
4. Cod-liver oil	*0·65*	1·0	*0·6*	*0·6*	*0·55*
5. Castor oil	*0·65*	*0·7*	*0·7*	*0·55*	*0·6*
6. Olive oil	*0·65*	1·0	*0·7*	*0·6*	*0·6*
7. Lecithin	1·45	1·8	1·3	1·5	1·75
8. Lanolin	*0·75*	1·0	*0·6*	0·65	*0·7*
9. Beeswax	*0·75*	1·5	*0·55*	*0·55*	*0·5*
10. Cholesteryl stearate ...	*0·7*	1·1	*0·45*	*0·4*	*0·6*
11. Cholesteryl palmitate ...	*0·7*	1·1	*0·55*	*0·35*	*0·6*
12. Cholesteryl propionate ...	*0·7*	1·1	*0·55*	*0·4*	*0·6*
13. Carnauba wax	*0·75*	—	*0·55*	*0·55*	*0·65*
14. Chinese wax	*0·8*	—	*0·65*	*0·55*	*0·65*
15. Spermaceti	1·0	—	*0·65*	0·75	0·75
Gland control	*0·65*	*0·6*	*0·5*	*0·35*	*0·45*

Thymus.

	Substrate	Ox	Sheep	Pig
1.	Butyrin	3·6	5·1	2·1
2.	Palmitin	1·2	1·0	1·4
3.	Stearin	1·0	1·0	1·0
4.	Cod-liver oil	1·4	1·1	1·1
5.	Castor oil	1·4	1·1	0·9
6.	Olive oil	1·7	1·2	1·1
7.	Lecithin	2·7	2·7	3·0
8.	Lanolin	2·0	1·1	1·0
9.	Beeswax	1·7	1·3	1·0
10.	Cholesteryl stearate ...	1·8	1·2	0·9
11.	Cholesteryl palmitate	1·4	1·2	0·8
12.	Cholesteryl propionate	1·0	1·2	1·0
13.	Carnauba wax ...	0·9	0·9	0·8
14.	Chinese wax	0·8	1·0	0·8
15.	Spermaceti	1·5	1·0	0·8
	Gland control ...	*0·3*	*0·5*	*0·5*

Liver.

	Substrate	Maximum acidity	Human	Ox	Sheep	Pig	Cat	Rabbit	Guinea-pig
1.	Butyrin	19·9	4·0	12·4	8·5	16·0	14·0	16·0	16·0
2.	Palmitin	7·2	2·0	1·6	*0·2*	*0·5*	1·3	2·0	0·9
3.	Stearin	6·2	1·8	1·6	*0·3*	*0·4*	1·0	2·0	*0·4*
4.	Cod-liver oil	6·7	2·0	2·8	*0·4*	0·8	1·0	2·0	1·15
5.	Castor oil	6·6	2·0	2·9	*0·4*	1·0	1·0	2·0	1·0
6.	Olive oil	7·0	2·0	2·9	*0·5*	1·0	1·0	*1·7*	1·9
7.	Lecithin	—	6·9	5·5	*5·5*	6·8	4·8	6·0	3·3
8.	Lanolin	7·0	*1·2*	2·4	*0·4*	1·4	1·2	2·0	*0·4*
9.	Beeswax	6·7	2·1	2·0	*0·3*	1·2	*0·9*	2·0	*0·8*
10.	Cholesteryl stearate ...	6·2	1·8	2·4	*0·3*	1·2	1·1	2·0	*0·5*
11.	Cholesteryl palmitate	9·0	1·8	2·2	*0·4*	1·0	*0·8*	2·0	*0·5*
12.	Cholesteryl propionate	9·0	1·8	2·4	*0·5*	1·2	1·0	2·0	*0·5*
13.	Carnauba wax ...	5·6–6·8	*1·2*	1·4	*0·4*	0·8	*0·8*	—	*0·5*
14.	Chinese wax	—	*1·2*	1·4	*0·3*	0·7	1·1	—	*0·5*
15.	Spermaceti	8·8–9·6	*1·4*	2·6	*0·5*	1·2	1·3	—	*0·6*
	Gland control ...	—	*1·3*	*0·3*	*0·3*	*0·4*	*0·6*	*1·5*	*0·5*

Spleen.

	Substrate	Human	Ox	Sheep	Pig	Cat
1.	Butyrin	1·0	0·8	0·5	1·8	1·5
2.	Palmitin	*0·3*	*0·6*	*0·3*	*0·8*	*0·8*
3.	Stearin	*0·25*	*0·6*	*0·3*	*0·75*	*0·8*
4.	Cod-liver oil	*0·35*	*0·5*	*0·3*	1·25	*0·8*
5.	Castor oil	*0·35*	*0·4*	*0·3*	1·25	*0·8*
6.	Olive oil	*0·35*	0·7	*0·5*	1·7	*0·8*
7.	Lecithin	4·3	2·4	2·3	7·0	5·0
8.	Lanolin	*0·3*	*0·4*	*0·4*	*0·6*	*0·7*
9.	Beeswax	*0·3*	0·8	0·7	*0·8*	*0·8*
10.	Cholesteryl stearate ...	*0·3*	*0·4*	*0·3*	*0·7*	*0·6*
11.	Cholesteryl palmitate ...	*0·3*	*0·4*	*0·3*	*0·7*	*0·6*
12.	Cholesteryl propionate ...	*0·3*	*0·4*	*0·4*	*0·7*	*0·6*
13.	Carnauba wax	*0·3*	—	—	—	*0·5*
14.	Chinese wax	*0·3*	—	—	—	*0·6*
15.	Spermaceti	*0·3*	—	—	—	0·9
	Gland control	*0·3*	*0·4*	*0·3*	*0·7*	*0·6*

Kidney.

Substrate	Human	Ox	Sheep	Pig	Cat
1. Butyrin ... ... (fatty)	6·65	0·8	0·9	—	4·0
2. Palmitin	2·2	*0·3*	*0·4*	—	0·65
3. Stearin	2·2	*0·3*	*0·4*	—	0·55
4. Cod-liver oil	2·5	*0·4*	*0·4*	—	0·6
5. Castor oil	2·4	*0·4*	*0·4*	—	0·6
6. Olive oil	*1·8*	*0·4*	*0·4*	—	0·7
7. Lecithin	2·5	1·05	1·2	—	1·35
8. Lanolin	2·2	*0·4*	*0·4*	—	*0·4*
9. Beeswax	*2·0*	0·6	*0·5*	—	*0·45*
10. Cholesteryl stearate ...	3·0	*0·3*	*0·4*	—	*0·45*
11. Cholesteryl palmitate ...	2·4	*0·3*	*0·4*	—	*0·45*
12. Cholesteryl propionate ...	2·2	*0·4*	*0·4*	—	*0·45*
13. Carnauba wax	—	—	—	—	*0·35*
14. Chinese wax	—	—	—	—	0·55
15. Spermaceti	—	—	—	—	0·6
Gland control	*1·8*	*0·3*	*0·4*	—	*0·25*

Thyroid.

Substrate	Ox	Sheep	Pig
1. Butyrin	1·6	4·3	0·4
2. Stearin	0·9	*0·1*	*0·1*
3. Cod-liver oil	0·7	0·4	*0·15*
4. Castor oil	0·8	*0·2*	*0·1*
5. Olive oil	0·8	*0·3*	*0·1*
6. Lecithin	3·3	2·3	0·85
7. Lanolin	1·0	0·4	*0·1*
8. Beeswax	0·9	0·4	*0·1*
Gland control ...	*0·4*	*0·1*	*0·1*

Skins. Bile. Salivary glands.

Substrate	Skins, human 1	2	3	Bile, Ox	Salivary glands, Pig
1. Butyrin	6·5	2·1	—	3·5	*0·55*
2. Palmitin	4·2	0·7	—	*0·5*	*0·25*
3. Stearin	4·0	1·1	—	*0·5*	*0·2*
4. Cod-liver oil	3·5	0·65	—	*0·5*	*0·25*
5. Castor oil	3·0	0·65	—	*0·5*	*0·3*
6. Olive oil	*0·2*	0·65	—	*0·8*	*0·3*
7. Lecithin	1·6	1·0	—	*0·5*	1·3
8. Lanolin	3·8	0·9	0·35	*0·5*	*0·2*
9. Beeswax	4·5	1·0	0·35	*0·5*	*0·2*
10. Cholesteryl stearate ...	4·7	1·0	0·4	*0·5*	*0·2*
11. Cholesteryl palmitate ...	5·0	0·85	0·35	*0·5*	*0·25*
12. Cholesteryl propionate ...	4·2	0·75	0·35	—	*0·2*
13. Carnauba wax	—	0·75	0·45	—	*0·2*
14. Chinese wax	—	0·7	0·4	—	*0·2*
15. Spermaceti	—	0·75	0·45	—	*0·25*
Tissue control	*0·2*	*0·15*	*0·05*	*0·5*	*0·2*

	Substrate	*Pituitary.* Ox	*Pituitary.* Sheep	*Bone marrow.* Ox
1.	Butyrin	0·8	*0·4*	3·4
2.	Palmitin	*0·65*	*0·1*	*0·8*
3.	Stearin	*0·55*	*0·1*	*0·8*
4.	Cod-liver oil	0·75	*0·1*	*0·8*
5.	Castor oil	*0·5*	*0·1*	*0·7*
6.	Olive oil	0·7	*0·1*	*0·7*
7.	Lecithin	1·2	0·85	1·8
8.	Lanolin	*0·5*	*0·2*	*0·7*
9.	Beeswax	*0·55*	*0·15*	*0·8*
10.	Cholesteryl stearate...	*0·45*	*0·2*	—
11.	Cholesteryl palmitate	*0·55*	*0·25*	—
12.	Cholesteryl propionate	*0·5*	*0·25*	—
13.	Carnauba wax ...	*0·5*	*0·1*	—
14.	Chinese wax	*0·65*	*0·1*	—
15.	Spermaceti	*0·5*	*0·15*	—
	Gland control ...	*0·3*	*0·1*	*0·6*

Conclusions.

Fat- and wax-splitting ferments are widely distributed among the organs of man, ox, sheep, pig, cat, rabbit, and guineapig.

Butyrinase and lecithase were present in all of the 58 organs examined, and were often very strong.

Olein lipase was also commonly present, but often as a mere trace. It was strongest in pigs' pancreas, but also active in the pancreas of other species, in the liver, and in the thymus, and to a lesser degree in lymphatic glands, ox suprarenals and thyroid, cat kidney, and pig lung.

Tristearin ferment was less frequently found, but was active in the ox, sheep and pig pancreas, the human, ox, cat and rabbit liver, the thymus, ox and sheep lymphatic glands, the human skin, human and cat kidney, ox suprarenals, and ox thyroid.

Wax-splitting ferments were found in a variety of organs, ox, sheep and pig pancreas, human, ox, pig, cat and rabbit liver, thymus, lymphatic glands, ox and sheep thyroid, ox suprarenals, human skin, and cat and human kidney.

The distribution of esterases in the body appears to have some relation to resistance to tubercle. The cat, which is very little susceptible to tubercle, is well provided with esterases throughout its organs. The sheep, although less well provided for than the susceptible pig in other regions, possesses very active lymphatic glands. The susceptible guineapig has the chief part of its

lipase in the form of butyrinase and olein-lipase, and is deficient in higher esterases. The lungs in all species were found very poor in these ferments. The more resistant liver shewed active lipolytic properties.

I desire to express my thanks to Dr Cramer for his kind advice and encouragement. The expenses of this research were met in part by a grant from the Earl of Moray Trust.

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XLII. THE GROWTH OF RATS UPON DIETS OF ISOLATED FOOD SUBSTANCES.

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In a recent communication on the growth of rats upon artificial diets containing lactose, published in this journal, Drummond [1916] has discussed a number of problems with which we, as well as other investigators, have been concerned for several years. The facts reported are essentially in harmony with our own experience in the study of the growth of white rats fed upon mixtures of isolated food substances. Inasmuch as readers of Drummond's paper who have not carefully followed our own publications might easily gain the impression that our views are at variance with his conclusions as well as with those of some other workers in this field at the present time, we regard it as desirable to refer here to a few of the topics involved.

In order to appreciate some of the changes of opinion which have taken place as the result of more recent studies in feeding animals on isolated food mixtures, it should be pointed out that earlier we have recorded experiments showing successful growth for such considerable periods that it seemed reasonable, at that time, to conclude that the diets employed were fully adequate for prolonged nutrition. Subsequent and more extended experience demonstrated, however, that failure and decline of body weight inevitably ensued unless additional accessory substances were furnished in the diet. By the use of what we termed "protein-free milk," along with isolated proteins, starch, and fats in the form of lard, we usually succeeded in promoting the growth of albino rats for about eighty days; and when the failure on

these diets manifested itself, the decline was averted by the substitution of certain natural fats, notably butter fat, for a part of the lard. This agrees with the experience of other investigators in this field. The inevitable ultimate failure of all experiments in which a fat-soluble accessory component was missing was at first overlooked, owing to the fact that the earlier observations were made over too short a period of time to permit the nutritive decline to manifest itself. This statement applies to the results of the feeding experiments with fat-free food mixtures which we published in 1912 [Osborne and Mendel 1912]. We have not claimed, however, that the diets in these experiments were "*lipoid*"-free. We considered them as evidence of the possibility of very considerable constructive nutrition in the absence of the ordinary fats. Indeed, some time ago we attempted to avoid any misconception regarding our previous experience by the following statement: "In reviewing our earlier published experiments Funk has erroneously assumed that we secured completed growth with the diets in which the butter component was not yet employed. It is true that the increments in weight were in some cases very noteworthy; but in every instance cessation ultimately ensued before the completion of the normal progress of the growth or subsequent maintenance. We have never denied the necessity of a growth-promoting food accessory in accord with the claim of Hopkins; and recently we pointed out that the successful partial completion of growth, such as has been obtained in our experiments, may well have been due to a store of the essential compound in the body of the experimental animals at the beginning of the trials. It is by no means necessary to assume with Funk that small quantities of these accessory substances were inadvertently left in our food preparations owing to insufficient extraction with alcohol" [1913, 2, p. 430].

After our discovery that "protein-free milk" together with starch, butter fat, and an adequate protein furnishes a suitable ration for rats, we imitated the inorganic ingredients of the "protein-free milk" by a suitable combination of bases and acids,—our "artificial protein-free milk." When this was used in place of the natural product we obtained considerable growth in several instances; but in the great majority of cases little, if any, growth was made. McCollum and Davis [1915] also have reported not a few examples of very considerable growth upon similar rations consisting of isolated food substances and artificial salt mixtures. The possibility of obtaining adequate growth in this way is very slight; and it is always merely a question of time before a nutritive decline ensues unless a change is instituted in the ration,

whereby the lacking accessory substances are furnished. Whether the growth occasionally made on these purely artificial mixtures is caused by impurities attached to the lactose used—as has been intimated by Drummond—or whether certain animals are possessed of a sufficient store of nutrition-promoting "hormones" to enable them to utilise highly purified isolated food substances in a constructive way for some time, is debatable in view of the very small proportion of rats which make any material growth of such foods.

Our attitude was summarised in 1913 as follows: "Our own experiments, as well as those of the other investigators mentioned, make it clear that something further than a sufficient supply of energy-yielding food material is required to promote a normal growth. The animal cells need for their activities not only energy, but also suitable constructive material to replace the wear and tear therein. Furthermore the cells are concerned in the elaboration of a great diversity of complex and little understood substances such as enzymes, products of internal secretion, etc., which unquestionably play an indispensable rôle in life and may require either special antecedent products for their construction, chemical activators of some sort, or minute quantities of readily overlooked rarer elements and compounds. It is easy, yet futile at the present time, to develop detailed hypotheses respecting the almost innumerable possibilities involved. The greatest promise of success in discovering the food factors which determine successful growth lies in seeking them in some chemical constituents of such diets as have proved adequate to promote growth.

"In attempting to ascertain what constitutes an adequate diet, by feeding experiments with isolated substances, we have found that our purely artificial foods—mixtures of isolated proteins, fats, carbohydrates and inorganic salts—sooner or later fail to maintain mature animals. In view of this such dietaries may naturally be expected to fail to maintain the less resistant young during their adolescent period. It is true that in several instances we have succeeded in keeping grown rats in health and in apparent nutritive equilibrium on purely artificial food mixtures over periods far longer than the experience of our predecessors had led us to expect. But the outcome has never been satisfactory in the sense of extending over what may be considered as the larger portion of the life span of an adult animal. Successful maintenance has been secured only when the animals were fed, in part at least, with foods containing our 'protein-free milk,' the preparation and composition of which has been detailed elsewhere. The superiority of the

latter foods, compared with any purely artificial food mixture in repairing the depleted body weight of animals that have begun to decline on the artificial salt mixtures tested, is beyond question. Instances of immediate recovery following the replacement of the inorganic constituents of the dietaries and part of the carbohydrates by the 'protein-free milk' have been published, and might be duplicated in great numbers from our protocols. Even greater success is manifested in maintenance experiments in which the 'protein-free milk' alone furnished the inorganic constituents of the dietary during long periods of time. The superiority of the 'protein-free milk' foods over the diets containing artificial salt mixtures in the maintenance experiments also is unquestioned. Wherein the difference lies is not yet apparent. We have already pointed out that the efficiency of this adjuvant to the energy-yielding nutrients is not attributable to the minute trace of milk protein present. The fact of the greater efficiency of the natural milk product suggests that some constituent present in milk is essential for prolonged maintenance" [1913, 1, p. 312].

As a result of the circumstances here recorded we were led, like McCollum and Davis, to investigate the fat-soluble components (McCollum's fat-soluble A) that promote growth in the presence of other suitable dietary ingredients.

Similarly one of us has recently stated [Mendel 1915]: "It is not unlikely —to speak conservatively—that there are at least two 'determinants' in the nutrition of growth. One of these is furnished in our 'protein-free milk' which insures proper maintenance even in the absence of growth. When this was fed we have maintained rats without growth for very long periods. Without this 'determinant' (as, for example, in diets of isolated food substances containing artificial substitutes for 'natural' protein-free milk) the special components of butter fat or cod-liver oil or egg fat induce only limited gains at best. Another 'determinant' is furnished by these natural fats (or fractions more recently prepared therefrom by Osborne and myself or the saponification product of McCollum and Davis). Either of the determinants may become 'curative'; both are essential for growth when the body's store of them (if such there be) becomes depleted. It is too early to attempt a tenable conclusion."

We believe the foregoing statements published by us some time ago should make it apparent that, like Drummond, we have concluded "before complete growth can occur in a young animal, the diet, besides being adequate as regards its proteins, carbohydrates, fats and salts, must contain both fat-soluble and water-soluble accessory substances" [Drummond 1916, p. 102].

Drummond has stated [1916, p. 93]: "Osborne and Mendel have always been careful to utilise, throughout their extensive researches, proteins of a very high degree of purity, and yet they have gone to the other extreme in continuing to use in their diets 'protein-free milk.' Reference has already been made to the criticisms which have been directed against the use of this complex substance, as a source of lactose and salts." In this connection the reader should be reminded that our primary object was the study of the relative nutritive value of certain proteins which can be undertaken only when *all* the other (non-protein) essentials of the diet are available. The use of "protein-free milk," to which exception has been made above, has proved valuable in that it has offered an unusually good source for the water-soluble "accessory," "vitamine," "determinant," or food "hormone" which, along with suitable fat-soluble accessory, has enabled us to conduct experiments, which otherwise would have been impossible, on the relative value of certain proteins and amino-acids[1]. For our *studies of the rôle of proteins in nutrition* it has been essential that all necessary food "hormones" should be included in the diet. Consequently if the lactose used actually contained traces of "vitamines," this would offer no disadvantage for the conduct of such experiments. Obviously this does not apply to the use of such a product for investigations of the nature of the food hormones or vitamines.

[1] The possible significance of the small quantity of protein present in our preparation of "protein-free milk" has not been overlooked. [See Osborne and Mendel, 1915.]

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XLIII. THE PECTIC SUBSTANCES OF PLANTS.

(Preliminary communication.)

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(Received November 14th, 1916.)

The substances known as the pectins, which are said to form the middle lamella of plant cells, offer many points of interest. It is supposed that they are readily attacked by various fungi which are the causes of plant diseases, and a knowledge of their chemistry is, therefore, of considerable importance to plant pathologists. They also, under certain conditions, which are not yet clearly understood, form gels, and it was mainly the interest in this particular property which first attracted the authors of this paper to their investigation. One of them (S. B. Schryver) had already commenced a systematic chemical research, when the other [Haynes 1914] published a paper dealing with the conditions under which pectic gels were formed, and they decided to continue in partnership their researches, which have, unfortunately, been repeatedly interrupted by other work.

Very little of the literature dealing with the subject of the pectins is of recent date, and the statements made by earlier authors are often confused and contradictory. It is not proposed to discuss the literature in any detail in this place, as it is dealt with in an adequate manner in various hand-books. (See more especially the works of Czapek [1913] and Tollens [1914].)

The materials employed by the authors for investigation comprised turnips, strawberries, rhubarb stem and apples, and the results so far obtained indicate that one single substance of acidic character is obtained from all sources; this has been designated *pectinogen*. On treatment with weak solutions of caustic alkalis at ordinary room temperature it readily undergoes conversion into a second substance to which the name *pectin* has been given from which gels can be readily obtained. It must be admitted that the

evidence as to the identity of the pectinogen from all the sources investigated cannot be considered as complete, but as relatively large amounts of material are now available for further work, it is hoped that subsequent researches which it is proposed to carry out in this laboratory, will lead to a definite conclusion as to this point. All the samples of pectinogen, however (which has not yet been isolated in a pure state) can be converted into pectins under the same conditions; the pectins from all sources, furthermore, give on combustion almost identical analysis numbers, which correspond very closely with a definite chemical formula containing seventeen carbon atoms (or multiples thereof); the furfural obtained by the distillation with hydrochloric acid of the pectinogen from two sources has also been estimated and the results indicate very definitely that each complex of seventeen carbon atoms contains one pentose group. The preliminary results point, therefore, to the conclusion that there is a single pectin which is a complex built up by the combination of a pentose group with another group containing twelve carbon atoms. There is no evidence that it is a carbohydrate, but it appears to be an acid *sui generis*. As several hundred grams have now been prepared, an opportunity exists for a more complete chemical research on the nature of this interesting substance.

Preparation of pectinogen.

The principle of the method employed is the same for all materials used, but it must be varied in details to suit their different textures. The expressed juices of the plant tissues appear to contain only small amounts of pectinogen, and only in the case of apples has any appreciable amount been obtained from such sources. It is generally extracted from the plant residues which remain after the expression of the juices by treatment with warm 0·5 % ammonium oxalate solution; from this extract after concentration to a small bulk it can be precipitated in a gelatinous form by the addition of acidified alcohol. By far the largest amount of pectinogen prepared in this laboratory has been obtained from turnips (or swedes) and these give the most satisfactory yield of all the materials hitherto investigated.

Preparation from turnips. The turnips are first coarsely disintegrated in a motor-driven mincing machine and the material thus obtained is placed in a cloth and pressed as rapidly and completely as possible in an ordinary tincture press; the juice collected, which contains no appreciable amount of pectinogen, is discarded. The residue is then mixed with acetone, a procedure which tends to stop fermentative action, and also facilitates the

subsequent drying processes. About 2¼ litres of acetone were mixed with the residue from about 40 lbs. of turnips (one Winchester quart to one bushel of vegetables). The moistened mass was then pressed again through the mincing-machine which was "set" to the small apertures so as to obtain the material in a finer state of aggregation. It was then wrapped in a cloth and pressed, first in a tincture press and then in a Buchner press at about 200 atmospheres pressure. The residue obtained in this way was then very rapidly dried (a process which takes a relatively short time) in a current of air at 37°, a "serum-drier" supplied by Hearsons having been employed for this operation. The powder obtained in this way was then finely ground by passage through a motor-driven grinding mill.

In one experiment 4730 g. of dry material were obtained from about 100 kilos of turnips.

The powder was then washed with cold water to free it from the last traces of soluble product. The amounts of water used for washing and of ammonium oxalate solution for the subsequent extraction of the pectinogen are so chosen that a paste is formed which can be enveloped in a cloth and pressed out afterwards in a tincture press. It is not always easy to do this, for the dried powder swells very considerably on the addition of water, and different powders absorb different amounts of the liquid. If the ratio of powder to water is too high, it is difficult to express the liquid in a press; if, on the other hand, too much water is added, a paste is obtained which is too thin to manipulate in the manner described. In this latter case large amounts of liquid must be used for washing and extraction and separated from the solid by decantation. By practice, however, such a contingency can be generally avoided.

Details of the experiment already mentioned will serve to illustrate the process. The 4730 g. of powder obtained from 100 kilos of turnips were washed twice with 24 litres of water, the washings being expressed each time with the tincture press. The moist residue was then divided into two approximately equal portions, each of which was extracted twice with hot 0·5 % ammonium oxalate solution. This solution was heated to 80–90° in an enamelled bath, and the moist powder was then thrown in and well stirred with a wooden spoon; the paste thus obtained was wrapped quickly in a cloth and pressed whilst hot in a tincture press, a viscid extract being thereby obtained; the second extract of the first portion was used for the first extraction of the second portion; altogether, including the solution used for washing the cloths, 24 litres of extract were obtained. This ammonium

oxalate extract was then concentrated *in vacuo* (temperature of water bath 40–45°) to about 8 litres. Chloroform and toluene were added to the liquids as soon as they were obtained, to prevent growth of moulds and bacteria which readily attack pectinogen solutions, and small amounts of these liquids were also added from time to time to the extracts during the course of their concentration. To the concentrated extract of 8 litres, about 2–3 times the volume of 95 % alcohol was added, and the pectinogen then separated in the form of a very voluminous gelatinous precipitate. This was filtered off on to blotting paper contained in large funnels, and when it had drained sufficiently it was removed from the filters, wrapped in a cloth and pressed. The cake thus obtained was then redissolved in water, a process which requires several hours and occasional vigorous stirring. The turbid aqueous solution obtained in this way was then filtered through blotting paper pulp on a large Buchner funnel. It was necessary to change the pulp several times in the course of the filtration as it soon became clogged. All the pulp used was collected and heated with water in a beaker immersed in a water-bath and the washings thus obtained were filtered off on to a Buchner funnel, the residue being well pressed and subsequently washed on the funnel. These washings were added to the filtrate, and the total bulk in this particular experiment amounted to 12 litres. The opalescent fluid thus obtained still contained small quantities of suspended particles which could be separated from the fluid by centrifugalisation. (This operation was generally omitted at this stage when large amounts of pectinogen were being prepared owing to the great bulk of the fluid. In most of the work, the crude pectinogen was purified in small quantities as required; for this purpose it was dissolved in water to yield about a 3–4 % solution which was then centrifuged; the liquid was then poured off from the deposit and the purified pectinogen was precipitated from the cleared fluid by alcohol.) The fluid was then evaporated to about 5 litres, rendered distinctly acid to Congo red by dilute hydrochloric acid, and the pectinogen was precipitated by the addition of about twice the volume of 95 % alcohol. The gelatinous precipitate was then filtered, washed on the filter with 75 % then with 95 % alcohol, pressed, and finally allowed to stand for some time under absolute alcohol. It was then filtered off and air-dried, and was thus obtained in the form of a granular white powder. Yield from 100 kilos of turnips 355 g., which on heating in a steam-oven lost nearly 25 % of its weight.

Preparation from strawberries. About 150 lbs. of strawberries, after separation of the stalks, were mashed up with about 10 litres of acetone.

The mash was filtered on to cloths, and the residue thus obtained was allowed to stand under alcohol and then pressed, first in a tincture press, then in a Buchner press; it was then dried at 37° in a current of air. The dry powder weighed about 1·5 kilos. It was washed 5 times by decantation with large quantities of water and then extracted, first with 24 litres and afterwards with 8 litres of warm 0·5 % ammonium oxalate solution. The extract was evaporated to 6 litres before precipitation with alcohol. The purification of the precipitate was carried out by the method described in the case of pectinogen from turnips. 150 lbs. yielded about 95 g. of air-dried product.

Preparation from apples. The apples were minced and pressed in a tincture press, the juice was concentrated to a small bulk and alcohol was added. The gelatinous precipitate thus obtained was filtered off, pressed and extracted with warm ammonium oxalate solution. From this the pectinogen was obtained by the method already described. The greater part was, however, obtained from the residue of the apples remaining after expression of the juice, by the method employed in the extraction of pectinogen from turnips. The total yield from apples was very small.

Preparation from rhubarb stem. 16 kilos of rhubarb stem were beaten into fibre by a wooden mallet and the disintegrated mass was pressed in a tincture press. The residue was treated with 4·5 litres of acetone, and then pressed again, first in a tincture and afterwards in a Buchner press. The material thus obtained was dried in an air current at 37° and then ground. The method of preparation of pectinogen from the powder obtained in this way was similar to that employed in the case of turnips. Yield, about 25 g. of air-dried pectinogen, which was of brownish colour.

Properties of pectinogen.

Pectinogen is precipitated from aqueous solution by alcohol in the form of a very bulky gelatinous mass, which after treatment with absolute alcohol and drying in air, is converted into a granular powder. It is generally somewhat coloured, the tint depending on the source from which it has been prepared; the product obtained from turnips is nearly white. It dissolves in water somewhat slowly, as a considerable amount of imbibition of fluid must occur before dispersion takes place. It forms viscous, somewhat opalescent solutions which are distinctly acid to phenolphthalein.

0·3 g. of air-dried pectinogen from turnip required 13·3 cc. N/10 acid

for neutralisation. (The pectinogen was in this case dissolved in excess of alkali and the excess was titrated back.)

One mol of acid was therefore equivalent to 225 g. of air-dried pectinogen.

A solution of pectinogen made neutral to phenolphthalein by caustic alkalis yields no precipitate on the addition of either dilute acid or a dilute solution of a calcium salt, and in this respect differs from pectin into which it is so readily converted by the method described below.

No method has yet been discovered for preparing pectinogen in a pure state. The product examined was generally obtained from the crude pectinogen by dissolving it in water to form a solution of about 3 %, centrifuging, reprecipitating from this by alcohol, and washing with alcohol of graded strengths up to absolute, in which it was allowed to stand for some time. Specimens obtained in this way generally contained about 3 % of ash after drying *in vacuo* at 100° over phosphorus pentoxide. All specimens used for analysis both of pectinogen and pectin were dried in this manner. Two samples, one from turnips and the other from apples, yielded the following numbers on analysis:

0·2348 g. turnip pectinogen,	0·1129 g. H_2O,	0·3458 g. CO_2,	0·0081 g. ash
0·1420 g. apple ,,	0·0729 ,,	0·2153 ,,	0·0042 ,,

	H	C	O	Ash
Turnip pectinogen	5·53	41·60	52·87	3·33
Apple ,,	5·88	42·61	51·51	2·96

The H, C and O numbers are calculated for the ash-free product. They do not differ very markedly from those obtained for pectin, which, there is reason to believe, was obtained in a purer state.

The conversion of pectinogen into pectin.

As mentioned above, pectinogen rapidly undergoes a change when it is kept in alkaline solution at room temperature. A freshly prepared neutral solution gives a precipitate with neither acid nor a dilute solution of a calcium salt. If however it is made alkaline, a change is indicated by the fact that both the above-mentioned reagents will after a short time yield bulky gelatinous precipitates when added to the solution. A gelatinous precipitate is also formed when a pectinogen solution is allowed to stand in the presence of excess of lime-water. There is little doubt that pectinogen is rapidly converted by alkalis into another acid, pectin, which is insoluble in water and gives an insoluble calcium salt. The rate of change from pectinogen into pectin is very rapid and is nearly complete in a few minutes in the presence of normal sodium

hydroxide at room temperature. The pectin can then be precipitated as a gel on the addition of acids. It was generally prepared from a 2 % solution of pectinogen which was made distinctly alkaline and then allowed to stand over night at room temperature. From this solution the pectin was precipitated by the addition of an equal volume of concentrated hydrochloric acid, which was used rather than dilute acid as it dissolved out the greater part of the constituents of the ash (see analyses below). The gelatinous precipitate was filtered on to a Buchner funnel by a pump, washed with water till the washings were free from chlorine, and then with alcohol, and finally it was allowed to stand over night under absolute alcohol. It was dried *in vacuo* at 100° over phosphorus pentoxide before being submitted to analysis. It is soluble in alkali and is reprecipitated from the alkaline solution on addition of acids. The yield of pectin was generally about 60 % of the amount of pectinogen taken, but it was found that it is appreciably soluble in strong hydrochloric acid solution. Detailed research, which need not be described here, failed to reveal any other product than pectin, when pectinogen is acted upon by alkalis under the conditions described.

Pectinogen solutions also yield a gel (presumably also pectin) when allowed to stand with concentrated hydrochloric acid at room temperature. The rate of change is however much slower than that which takes place in alkaline solution.

Pectin is soluble in alkalis, and it was found that 0·3 g. of substance dried *in vacuo* at 100° over phosphorus pentoxide neutralised 15·6 cc. N/10 sodium hydroxide. It is not possible from this result, taken in conjunction with the numbers obtained from combustion, to draw any conclusions as to the basicity of pectin, owing to the probability of lactone formation. This point will form the subject of subsequent researches.

Analyses.

Turnip pectin :							
*0·1857 g.	0·0812 g.	H_2O,	0·2793 g.	CO_2,	0·0040 g.	ash
0·2026 „	0·0948	„	0·3112	„	0·0005	„
Strawberry pectin :							
*0·1677 g.	0·0770 g.	H_2O,	0·2545 g.	CO_2,	0·0022 g.	ash
*0·1518 „	0·0693	„	0·2294	„	0·0020	„
0·1942 „	0·0890	„	0·2963	„	0·0004	„
0·1764 „	0·0807	„	0·2693	„	0·0004	„
Rhubarb pectin :							
0·1834 g.	0·0892 g.	H_2O,	0·2813 g.	CO_2,	0·0010 g.	ash
0·1842 „	——		0·2815	„	0·0009	„
0·1860 „	0·0864	„	0·2857	„	0·0010	„
Apple pectin :							
0·2078 g.	0·0966 g.	H_2O,	0·3143 g.	CO_2,	0·0012 g.	ash
0·1678 „	0·0726	„	0·2536	„	0·0009	„

Percentages.

	C	H	O	Ash
Turnip	41·92	4·97	53·11	2·15 *
	41·99	5·21	52·80	0·25
Strawberry	41·94	5·17	52·89	1·31 *
	41·95	5·14	52·91	1·32 *
	41·70	5·10	53·20	0·21
	41·73	5·10	53·17	0·22
Rhubarb	42·06	5·43	52·51	0·54
	41·88	—	—	0·49
	42·12	5·19	52·69	0·54
Apple	41·49	5·18	53·33	0·58
	41·44	4·83	53·73	0·54

The analyses marked with an asterisk were made from samples of pectin which were precipitated from alkaline solution by dilute acid; it will be noticed that in all these cases the ash content is high. The carbon and hydrogen numbers are all calculated on the ash-free product.

As the mean of the above numbers excluding those of the apple pectin, the percentages found are C = 41·93, H = 5·17, O = 52·90. These are in agreement with the formula $C_{17}H_{24}O_{16}$, which requires C = 42·15, H = 4·96, O = 52·89. The numbers for carbon obtained in the pectin sample from apple are a little too low (about 0·5 %), but the other numbers agree in a most satisfactory way with the suggested formula.

Pectin from all sources is soluble in alkali and if strong sodium hydroxide is added to such a solution, the sodium salt is precipitated in the form of a gel. The neutral solution of pectin gives a gelatinous precipitate with calcium chloride.

Estimation of the pentose group in pectinogen.

Pectin itself is insoluble in acids and on heating with them readily undergoes charring. For this reason, the estimation of the pentose groups was carried out with the pectinogen, which is soluble in water, although no method has yet been discovered for purifying the crude product. The specimens employed were dried *in vacuo* at 100° over phosphorus pentoxide, after they had been precipitated from aqueous solution which had been centrifugalised. Both the turnip and rhubarb pectinogens contained 3·4 % of ash. They were heated with hydrochloric acid and the furfural which was evolved was estimated by the Tollens phloroglucinol method. The percentage is calculated on the weight of the ash-free product.

I.	0·3122 g. turnip pectinogen,	0·1114 g. phloroglucinide	= 0·0605 g.	furfural
II.	0·2935 „ „	0·1086 „	= 0·0590 „	
III.	0·2784 rhubarb „	0·0912 „	= 0·0500 „	

Percentage of furfural from turnips = (I) 20·1 and (II) 20·8
„ „ „ rhubarb = (III) 18·6.

A substance of the formula $C_{17}H_{24}O_{16}$ should yield 19·8 furfural, supposing it to contain one pentose group. As these estimations were carried out with crude pectinogens, the results are as near as could be expected and form strong evidence that each $C_{17}H_{24}O_{16}$ complex contains one pentose group.

Summary.

(1) A method is described for the preparation of the pectic substances of plants, in which the essential operation is the extraction of the residue, obtained after the expression of the juice, with a warm 0·5 % ammonium oxalate solution.

(2) A substance of acidic character is thereby obtained, which is soluble in water and is designated *pectinogen*.

(3) This on keeping in alkaline solution at room temperature readily undergoes conversion into another substance, also of acidic character, which is precipitated from its alkaline solution by acids as a gel insoluble in water. To this substance the name *pectin* has been given. It differs from pectinogen in that it is insoluble in water, and that the solution of its sodium salt gives a gelatinous precipitate with calcium chloride. A solution of pectinogen also gives a gel when allowed to stand at room temperature with an excess of calcium hydroxide solution. In this case, presumably a calcium salt of pectin is obtained. [See however Haynes, 1914]

(4) Pectins from turnips, strawberries, rhubarb stem and apples have been prepared. The analyses agree well with the formula $C_{17}H_{24}O_{16}$. Only in the case of the apple pectin were numbers for the carbon obtained which were slightly too low.

(5) Pectinogen on distillation with hydrochloric acid yields furfural in such a quantity as to indicate that one pentose group is contained in each complex of seventeen carbon atoms.

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XLIV. NOTE ON THE COLOURING MATTER OF RED TORULAE.

By ALFRED CHASTON CHAPMAN.

(*Received November 15th, 1916.*)

Some years ago I undertook in conjunction with Mr F. G. S. Baker, an investigation of the red torulae with the object of isolating as many distinct species as possible, and of obtaining results which might serve as a basis of classification. In connection with this work, during which six different species were obtained and studied, I commenced a subsidiary investigation of the red colouring matter present in these organisms.

One of the six species of torulae above referred to was grown on large plates containing wort-agar. When a sufficient quantity had accumulated the growth was scraped off, care being taken to remove as little of the agar surface as possible. It was then dried at a low temperature and was thoroughly ground with fine sand. The resulting material was then extracted with various solvents, chloroform and carbon disulphide giving the best results. In the case of both these solvents deep red solutions were obtained, which on cautious evaporation gave a coloured residue containing fat and probably some phytosterol. With the small quantities obtained the separation of these fatty substances was found to be impossible, and consequently no crystals could be got. It was found at the commencement of the work that the solution in chloroform when warmed and exposed to the light quickly became colourless, a fact which suggested that the colouring matter might be allied to carrotene. The extracted substance behaved like carrotene in certain other respects, being practically insoluble in water, and dissolving in concentrated sulphuric acid with a deep blue colour. About the time the work had reached this stage, Schimon presented to the University of Munich, a dissertation dealing with certain red coloured lower fungi. He appears to have obtained the same general reactions for the extracted colouring matter,

and concluded that it was identical with carrotene. Prior to the publication of this dissertation, I had commenced a study of the absorption spectrum of the colouring matter, with the object of comparing it with that of carrotene, and I observed that the two were not by any means the same. The examination was first made in carbon disulphide solution, with a glass prism, and the spectra were photographed, using Wratton and Wainwright's "Panchromatic" plates. In the case of the colouring matter from the torula, the light passed between wave lengths 5900 to about 7000, whilst in the case of carrotene, a much larger amount of the yellow portion of the spectrum was visible, the range of wave lengths being from about 5500 to 7000. In view of these results, I decided to make an examination of the colouring matter with a quartz prism, so as to obtain some information in regard to the far violet. The source of light used consisted of a Nernst lamp, and the solution of the colouring matter was contained in a Baly tube permitting of a progressive increase in the thickness of the solution examined without changing the containing vessel. For each exposure the position of the sodium lines was recorded by means of a small reflecting prism attached to the instrument, and on each plate the copper arc was photographed as an additional aid to fixing the position of the bands. Solutions of pure carrotene (prepared from carrots) in carbon disulphide were compared side by side with corresponding solutions of the torula colouring matter. In both cases observations were made in thicknesses 1, 2, 3, 4, 5, 6, 8, 10, 13, 16, 20, 25, 32, 40, 50, and 63 millimetres. The strength of the carrotene solution was 5·3 milligrams per litre, and the solution of the torula colouring matter was made of such a strength that the intensity of the absorption bands was comparable with that of the carrotene bands. Wratton and Wainwright's "Allochrome" plates were used, as these are insensitive to red light of greater wave length than about 5890. These, however, show a thin absorption band in the green, due to the colouring matter used in the preparation of the plates. "Panchromatic" plates were found to be less suitable for the purpose. The resulting spectrographs were found to differ very considerably and in a manner which might have been anticipated from a consideration of the absorption spectra as observed with the glass prism. It was found that in the case of the colouring matter of the torula, practically the whole of the yellow is cut off in solutions of sufficient concentration, whereas even in very much stronger solutions of carrotene the whole of the yellow passes. Moreover the absorption band in the blue is much wider in the case of carrotene than in the case of the torula colouring matter.

It is clear from these observations, either that the colouring matter of the torula consists of some other substance than carrotene, or that it consists of a mixture of carrotene with some other colouring matter. The solubility of the torula colouring matter in light petroleum appears to indicate that it does not belong to the xanthophyll group.

My best thanks are due to Dr Monier-Williams and to my assistant, Mr Frederick T. Harry, for valuable help in connection with the preparation of the spectrographs.

XLV. FEEDING EXPERIMENTS WITH DEFICIENCIES IN THE AMINO-ACID SUPPLY: ARGININE AND HISTIDINE AS POSSIBLE PRECURSORS OF PURINES.

By HAROLD ACKROYD and FREDERICK GOWLAND HOPKINS[1].

From the Institute for the Study of Animal Nutrition, Department of Agriculture, and from the Biochemical Department, Cambridge.

(*Received* 19*th December, 1916.*)

We have abundant evidence to show that purine derivatives can be synthesised in the animal body when no preformed purine material is present in the diet, but the chemical events which are involved in the formation of tissue purines are yet unknown. It is of course possible that the synthesis of the purine ring may start at a low level from simple and remote precursors. Attention has been fixed for instance upon the interesting observation made several years ago by Knoop and Windaus [1905] who showed that 5-methyl-iminazole is formed when a solution of dextrose is exposed to sunlight in presence of the strongly disscciated compound $Zn(OH)_2$, $4NH_3$. We have here in effect the formation of the iminazole ring, an essential part of the purine structure, by the action of ammonia upon sugar. That this reaction is related to the chemical mechanism by which histidine and purines are formed in plants is highly probable. In the case of the animal, synthesis of such a type is perhaps less likely. Nevertheless the observations of Cathcart [1909], of Graham and Poulton [1913], and of Umeda [1915] have shown that when the diet is rich in carbohydrates the excretion of endogenous purines is greater than when fat predominates, yielding the suggestion that the former may be employed in synthesis.

It is by no means established however that such a synthesis from carbohydrate derivatives and unknown nitrogenous substances is the sole

[1] Several of the experiments described in this paper were made in 1914, the rest in 1915. My colleague has been long at the front, and in writing the paper I have been unable to consult him. He has had moreover no opportunity of reviewing the experimental results as a whole. If therefore it be held that the conclusions are not warranted by the facts I am alone responsible. F. G. H.

or chief origin for purines. We are at any rate justified in looking first to the food for a supply of more immediate precursors.

When some structural relation is evident between a food constituent and a tissue constituent it is justifiable to suspect that the former, and not some wholly unrelated substance, provides the immediate raw material for the manufacture of the latter. The recognition of such a chemical relation should, at any rate, lead to an experimental enquiry into the possibility of a metabolic relation.

The fact that histidine alone among the amino-acids from protein has a chemical connection with purines in that its molecular structure comprises the iminazole ring has led others to suppose that histidine might be the precursor of purines in metabolism. Abderhalden and Einbeck [1909] tested the point experimentally, but obtained negative results. In their experiments the administration of histidine produced no definite increase in the excretion of allantoin. The experiments were repeated later in conjunction with Julius Schmid [1910]. In the later work a starving dog was given on two occasions an isolated dose of 10 g. of histidine hydrochloride but no increase of allantoin followed.

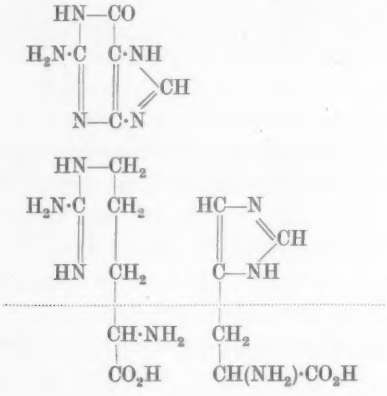
We venture to think however that these observers may not have secured the conditions necessary for properly testing the matter.

When an animal is in a state of full nutrition it does not follow that such a process as the synthesis of the purine ring would necessarily be much accelerated or increased by mere increase in the supply of its raw material. The accepted distinction between endogenous and exogenous metabolism and the recognised relative constancy of the former could scarcely hold were this the case. We know, it is true, that a large increase of pure protein in the diet does affect purine metabolism; but an individual amino-acid fed in excess of the immediate current needs of the tissues, as when it is added to an already efficient dietary, will almost certainly be rapidly broken down on more direct lines, even if it be a normal precursor of the purine (or other) synthesis in the body. We doubt, further, whether the administration of large isolated doses to a starving animal offers the best method for determining whether or not a substance suffers some special fate in metabolism. Doubtless synthesis of such essential tissue constituents as the purines continues during starvation, at the expense—as we are entitled to believe—of protein materials liberated by autolysis of the less essential organs. When however an excess of a single amino-acid enters the circulation of a starving animal in a single isolated dose it may well almost

completely escape such special utilisation. It appears suddenly in excess of current needs, and, because the processes of deamination and direct oxidation are always in action, it will almost certainly survive for but a short period as available material for synthesis. The increase in its concentration may momentarily accelerate the special reaction concerned, but the possible increase of reaction velocity in the tissue element is naturally limited, and the survival within the tissue cells of the abnormally increased amino-acid will probably be too short to affect appreciably the day's yield of a synthetic product. Much more satisfactory at any rate would seem to be experiments in which an animal, of which the normal metabolism is known, is first deprived entirely for considerable periods of the substance in question, and deprived of it alone. The effect of its absence being noted the substance can then be restored to the diet in normal amount and given continuously, when the results of its restoration can again be followed for considerable periods.

In the experiments to be described in this paper advantage was taken of the fact that from the complete mixture of amino-acids obtained upon hydrolysis of a typical protein, arginine and histidine can be efficiently removed by the well-known method of Kossel and Kutscher. Purine metabolism can thus be studied upon a dietary from which these two diamino-acids are absent and the effect of the deficiency as well as the result of restoring either or both can be observed.

The original incentive of the present research was, as a matter of fact, the idea that arginine and histidine might function together as precursors of the purine ring in metabolism, on some such lines as the following comparison of the structure of guanine with that of the two diamino-acids might suggest



Our original intention therefore was to test a possibility somewhat different from that which led to Abderhalden and Einbeck's experiments. During the progress of our observations however evidence was obtained which seems to show that in metabolism arginine and histidine are within certain limits equivalent to one another; equivalent probably because each can be converted into the other, just as phenylalanine can, up to a point at least, function in the place of tyrosine [Totani 1916] because in the body it can be converted into the latter (Embden). This circumstance removes some of the significance from the question as to whether both, rather than one only, of these two diamino-acids are concerned in the formation of the purine ring. The point however receives some further attention later on in this paper.

In most of our experiments, after a preliminary period during which a complete amino-acid mixture was administered, arginine and histidine were for some weeks removed from the diet, and then, in a third period, both were restored. The allantoin excretion was followed through each of these three periods.

In our opinion the results obtained offer good grounds for the belief that the molecules of arginine and histidine are as a matter of fact the most readily available raw material for the synthesis of purines in the body.

Methods.

The experiments were all made upon young growing rats. The rat when it has reached a weight of 80 to 100 g. continues to grow satisfactorily and for long periods on a diet which contains all its nitrogen in the form of a proper mixture of free amino-acids. It is certain however that successful feeding experiments when they involve the administration of abnormal diet can only be carried out on rats when care has been exercised in the choice of stock and when attention is given to the general care of the animals. Some stocks are more prone to disease, and especially to intestinal disorders, than are others, and, if such vulnerable animals are used, intercurrent disease may spoil a prolonged experiment just when it has reached its most interesting period. It is equally important that the experimental animals should be kept in healthy surroundings and at a uniform temperature. The cages should be kept clean and feeding should be carried out at regular intervals.

Admirably suited as is the rat for studies on growth it offers obvious difficulties when quantitative observations have to be made upon the urine.

The amount of urine is of course small and the proclivity of the rat when eating to hold food in its forepaws tends to scattering of the food and, in any ordinary collecting apparatus, to a contamination of the urine with the food.

The cages and apparatus used have been gradually modified by ourselves and by other workers in the laboratory and in their final form, as employed in the present research almost completely obviate any difficulty in collecting the urine quantitatively and with freedom from contamination. The

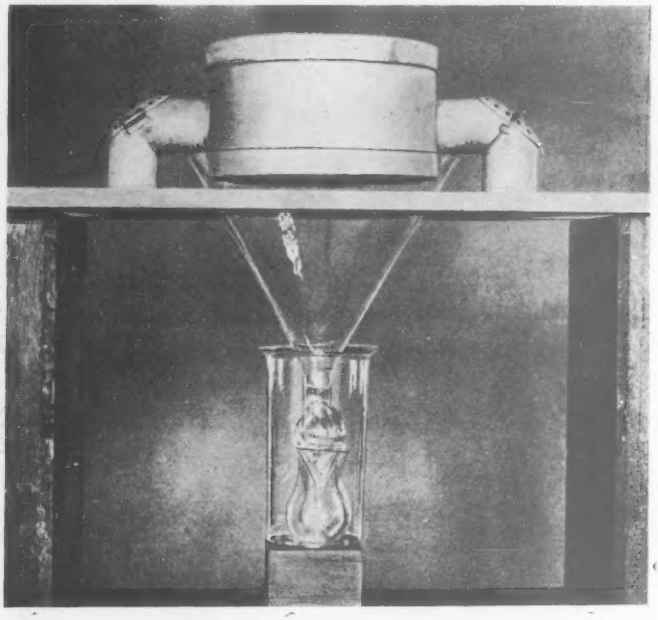


Fig. 1.

photographs illustrate the apparatus as used (Figs. 1 and 2). The cage itself is made of zinc. It is circular with a diameter of 10 inches, and a depth of $5\frac{1}{2}$ inches, and has a lid of perforated metal. The bottom consists of concentric circles of wire $\frac{1}{2}$ inch apart, held together by radial wires. The food and water are placed in glass vessels which fit into the vertical part of the metal side tubes. The tubes have a diameter of $2\frac{1}{4}$ inches. When the rat has to obtain its food under the conditions imposed by the tubes it seems unable to carry portions from the trough into the cage. This result

is more fully secured when the animal is wholly prevented from turning round while eating from the food-trough. For this reason inner tubes are provided, tapering somewhat towards their distal end. The diameter of these is adjusted to the size of the individual animals under experiment, and they are provided with radial flanges which slide tightly into the outer tube. Other details will be clear from the illustrations.

In our experiments no bedding was provided, as any material used is liable to soak up urine. The absence of bedding makes it important to

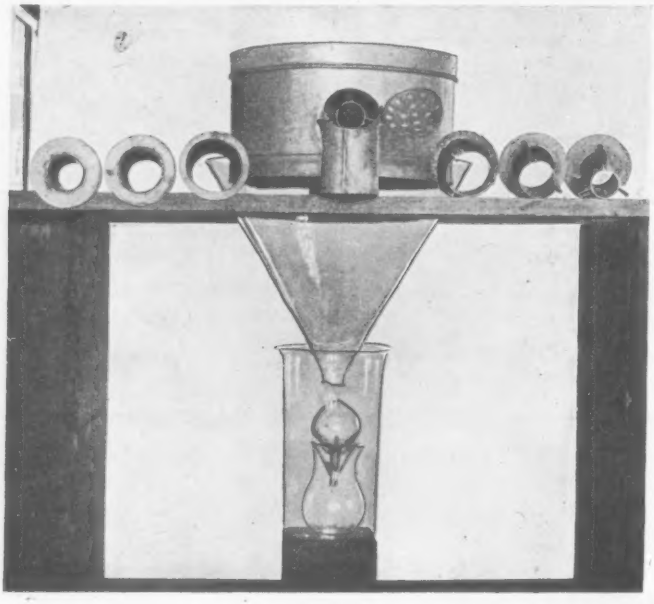


Fig. 2.

maintain the cages at a uniform temperature of about 65° F., and it is best that, as in our experiments, two animals should be fed in each cage. All our urine analyses represent the mean of the excretion of two rats.

For the collection of the urine we employed with slight modifications the apparatus first suggested by Paine and used by him at the Cancer Hospital, Fulham (Fig. 3). The metal cage stands within the upper part of a large glass funnel of somewhat larger diameter than its own. The short neck of the funnel reaches to within about $\frac{3}{4}$ inch of a glass globe.

This has a diameter of 2¼ inches and is provided with glass supports which allow it to stand in the neck of a small collecting vessel without touching the latter. An intermediary funnel as shown in the photographs may be used if necessary. The urine drops on to the globe without splashing and trickles over its surface into the collecting vessel whereas faeces and food particles are deflected and fall into an outer vessel. We have found it of advantage to give the glass globe a somewhat conical form as shown. The neck of the collecting vessel should have a diameter somewhat less than that of the globe.

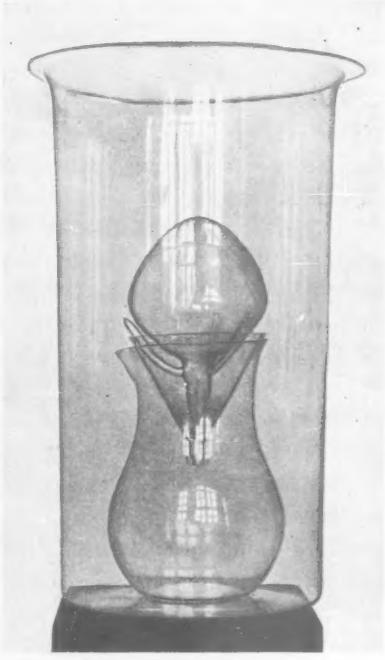


Fig. 3.

In each experiment the analyses were made upon the excretion of successive weeks. Shorter periods are unsatisfactory as their proper demarcation becomes impossible. The funnel in which the cage is placed was washed into the collecting vessel two or three times during the course of each day, small quantities of warm water being used. The urine and washings were transferred at frequent intervals to a stoppered bottle containing chloroform, the whole mixture being kept definitely acid throughout; acetic acid being added when necessary. During the week's collection the material was kept in an ice chest. At the close of the week it was made up to a standard volume for analysis.

For estimation of the allantoin Wiechowski's method was used. We first employed the method as originally described [1908] while in some of our later experiments the more recent modifications were used [Wiechowski, 1913]. In the case of rats' urine however we recommend the preliminary removal of ammonia by evaporation with magnesia and extraction with alcohol whatever modification of the process may be subsequently adopted.

As is well known the final stage of Wiechowski's process is reached either by determining the nitrogen in the mercury-allantoin precipitate, or by actually weighing the allantoin after its liberation from this compound. Wiechowski himself and especially Abderhalden and Einbeck look upon the latter procedure as the more trustworthy. This is not altogether in accord with our experience when working with diets containing free amino-acids. Sometimes a small amount of a syrupy non-nitrogenous substance is then apt to be present when the solutions are evaporated for the crystallisation of the allantoin. This however can be removed by the use of small quantities of ice-cold absolute alcohol. In several of our experiments both methods were used in every determination, and in the great majority of cases the agreement was exceedingly good. We finally came to rely with confidence upon Kjeldahl determinations of the nitrogen in the mercury precipitate. Although some of our experiments were made before the appearance of the paper by Givens [1914], in which he gives the experience of the Cornell laboratories bearing upon the conditions necessary for the stability of allantoin and other points of importance, we had fortunately observed just those precautions (in the preservation of the urine, etc.) which the paper indicates as necessary. Wiechowski's method is long and troublesome, and at no stage can it be entrusted to inexperienced hands. Its complexity limits the number of estimations possible for the individual worker and has prevented us from giving our results the statistical character for which feeding experiments upon small animals like rats otherwise afford the opportunity. As will be seen however our conclusions are based upon results got from a considerable number of individuals. We had hoped to carry out observations with the use of the method for estimating allantoin which has been proposed by Plimmer and Skelton [1914], but our leisure failed. The fault of that method lies in the fact that the allantoin nitrogen is determined by difference. Errors accumulate in estimating what is, after all, only a very small fraction of the total nitrogen. It is nevertheless very desirable that the method should receive full trial. In some experiments the total purine nitrogen of the urine was determined by the silver method of Camerer-Arnstein. Total nitrogen was estimated as usual by the Kjeldahl method.

The Diets Employed.

For experiments of this kind the standard control diet is prepared in the following manner (F. G. H.). Caseinogen, or better, a mixture of equal parts of caseinogen and lactalbumin is hydrolysed by boiling with 25 per cent. sulphuric acid for 20 to 30 hours. The sulphuric acid is quantitatively removed, and the fluid taken down to a syrup. At this stage a weighed quantity of potato starch, somewhat less than that meant to be present in the final diet is thoroughly incorporated with the syrup, and the resulting mixture spread out on a glass plate and allowed to dry in the air for two days. Tryptophane equal to 2 % of the protein originally taken for hydrolysis, and cystine equal to 0·5—1·0 %, are now added and thoroughly mixed with the whole. From this mixture, in which the nitrogen is determined, diets containing varying amounts of fat, additional starch, sugar, and mineral salts can be prepared with any desired nitrogenous content. Upon such diets well selected rats, if otherwise properly cared for, will show satisfactory, if not normal, growth, and will long remain in health. The effects of a tryptophane deficiency can be shown with great ease by refraining from adding that amino-acid to the hydrolysis mixture. Tryptophane certainly appears to be the only protein constituent of nutritive importance which is destroyed by acid hydrolysis, as the favourable results of its addition show. The addition of cystine is perhaps only of importance when the diet is given a low nitrogen content.

When in the present research the animals were to be fed in the absence of either arginine or histidine, or of both, the sulphuric acid of the original hydrolysis was reduced to about 1 %, and the solution of amino-acids well diluted (8 litres for 500 g. of original protein). It was kept at 40° and mixed with silver sulphate until sufficient was present to combine with all arginine and histidine (coloured precipitate on the addition of $Ba(OH)_2$). When working on a large scale this stage of Kossel and Kutscher's process occupies much time, but it is very essential that it should be properly carried out, and a full excess of silver added. The fluid was next saturated, while still at 40°, with powdered barium hydroxide. After cooling, the precipitate was filtered off at the pump and very thoroughly washed. From the bulky filtrate every trace of silver and barium was removed, and the whole evaporated to a syrup. In the preparation of the special diet this syrup was treated exactly as described above for the complete hydrolysis mixture. Tryptophane and crystine were added at the proper

stage, and if only one of the two diamino-acids was required to be absent a supply of the other was restored to the mixture. When the effect of restoring both to the food was under study the animals were not simply put upon a total hydrolysis mixture separately prepared, but arginine and histidine were restored to a mixture which had previously undergone treatment by the silver process, the results being thus more strictly comparable.

The silver method seems to remove both arginine and histidine with great completeness. If traces are left behind, and it is very difficult to disprove this possibility, the fact would not affect the bearing of our experiments, which were all comparative and controlled. The presence of traces in the diet of the experimental periods would tell against, rather than in favour of, our main conclusions.

The following example will illustrate the composition of the dietaries as fed:

Amino-acids from	200 g. of caseinogen
Potato Starch	250 „
Cane Sugar	125 „
Lard	45 „
Butter	45 „
[1]Ash from equal weights of Oats and Dog biscuit	21 „

This particular mixture contained 3·12 % of nitrogen; less than calculation from the original protein would indicate, but there is always some loss during the manipulation of the hydrolysis mixture. In some cases the diets have contained less nitrogen (down to 2 %) with proportionately more starch and sugar and fat. In each individual experiment the diet was identical throughout except for the qualitative variation in the amino-acid supply. Any change in purine metabolism was therefore independent of changes in the carbohydrates or fats.

The essential need of a vitamine supply comes to light with the greatest possible clearness when synthetic diets are prepared with amino-acids got by acid hydrolysis. Without a suitable addition there is no growth, and but short maintenance, when the assembly of amino-acids is complete. In our experiments this supply was given in the form of a protein-free alcoholic extract of fresh milk solids. Each rat received daily the equivalent

[1] There is no special reason for employing this particular mineral supply. It was originally used because oats and dog biscuits were the main food of stock rats in the laboratory. The ash has always proved satisfactory and large quantities were originally made so that its use has been continued.

of about 5 cc. of milk. This was true of every experiment described. To save printing we have not given the food consumption of our animals in the account of the experiments in the next section. We may state here that it was, throughout, sufficient to supply the energy not only for maintenance but for proper growth. This was true in the case of every experiment of which the protocol is given.

Experimental and Critical.

Effects upon Nutrition. Before giving the results of our allantoin estimations it will be convenient to deal separately with the effect upon body weight of the various dietetic deficiencies in connection with which we have studied the purine metabolism.

The pronounced effect upon nutrition produced by the withdrawal of tryptophane has, since the publications of Willcock and Hopkins, Osborne and Mendel, Abderhalden, Henriques and Hansen, and others, become well recognised. One of us (H.) has in various connections carried out a large number of experiments in which the nutritive condition of rats with and without tryptophane has been compared. Chart I summarises some of these results so far as the effect upon growth or body weight is concerned. The continuous lines show the effect of first withdrawing and of subsequently restoring tryptophane in the case of three separate rat pairs. The tryptophane was withheld on the 12th day (as shown in chart; the actual preliminary period was longer) of a diet containing a complete mixture of amino-acids. It was restored on the 35th day. The dotted lines show the average rate of growth upon the complete mixture (16 animals), and the average rate of loss in body weight when tryptophane is absent (8 animals). The average growth rate though satisfactory was not normal; but quite normal growth has been obtained in certain individuals when upon the complete mixture (hydrolysis products + tryptophane and a little cystine). On the other hand in a few cases not much more than maintenance without growth was attained [Asayama 1916] but this appears to have been due to deficiency in cystine as the result of prolonged hydrolysis of the protein, or to lack of adjustment in the vitamine supply. These cases were not included in the group yielding the average shown. After short hydrolysis of the protein (6 to 8 hours) maintenance, and some slight growth, has been observed in a few individuals, when no tryptophane was added to the hydrolysis products. Under these circumstances a certain amount of tryptophane escapes destruction and though certainly small it makes itself felt in nutrition. In all the

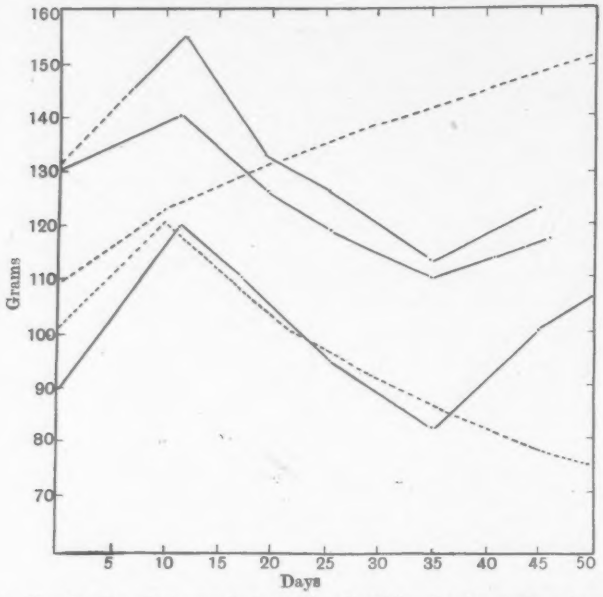


Chart I. Showing the effect upon body weight of withdrawing tryptophane from the diet. Continuous lines show mean weight of two rats in each case. Tryptophane removed on 12th day, restored on 35th day. The ascending dotted line shows average growth of 16 rats on complete amino-acid mixture. Lower dotted line average loss of 8 rats deprived of tryptophane.

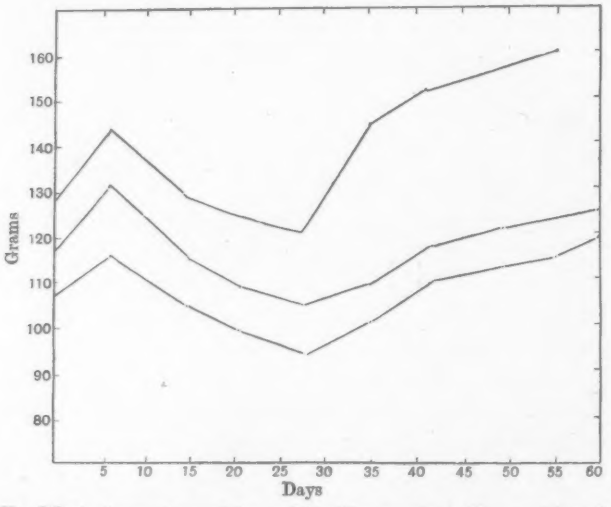


Chart II. Effect of removing arginine and histidine together. Mean weights of rat pairs. Diamino-acids removed on 7th day and restored on 28th day.

experiments yielding the results shown in Chart I the hydrolysis of the protein was continued for 24 hours or more.

When arginine and histidine are together removed from the products of protein hydrolysis the residual amino-acids are unable to support the animal. Chart II shows the effect upon body weight which the removal and restoration of these two diamino-acids respectively produce. When the removal has been as complete as possible the weight of animals taking the diet falls

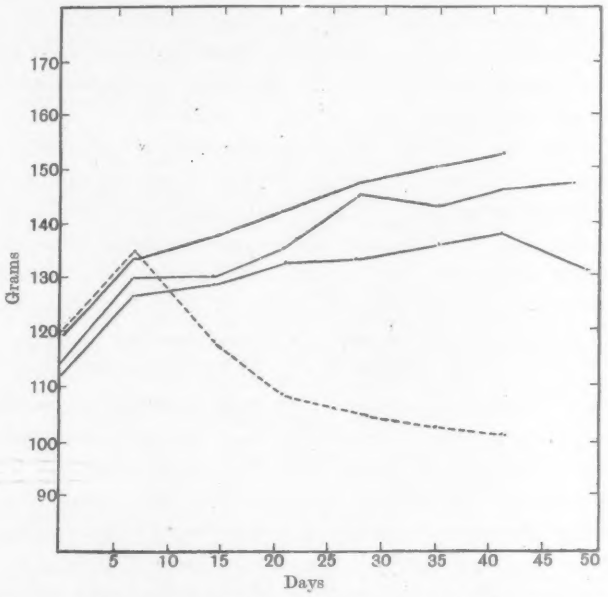


Chart III. Dotted line, body weight changes in the absence of both arginine and histidine. Lowest continuous line shows result when, of the two, only arginine was present. Two upper curves give weights when only histidine was present. Average weights of two rats in each case.

sharply. We have found however that this decline in weight becomes after a time less rapid than in the case of the tryptophane deficiency. There seems to be some adjustment to the lack of arginine and histidine. True, it must not be forgotten that, while prolonged hydrolysis destroys every trace of tryptophane, Kossel and Kutscher's method may leave behind minute amounts of the diamino-acids. The adjustment may be, not to the absence of arginine and histidine, but to the utilisation of a minute amount, sufficient for maintenance at a certain level, though not for growth. We believe

nevertheless that our preparations were free from these substances and that there are as a matter of fact different degrees of urgency in the need of an animal for a supply of this or that individual amino-acid in its food. On this assumption one of us has elsewhere briefly discussed the bearings of the present case [Hopkins, 1916].

The observations now to be mentioned illustrate a somewhat different aspect of the question as to how far the animal can dispense with individual protein units. They appear to us to yield an instance showing that the body can with some efficiency utilise one amino-acid vicariously for another.

The circumstance came to light when to the amino-acid mixture previously deprived of arginine and histidine, one, and only one of these two diamino-acids was restored.

The dotted line of Chart III shows once more the results when both are absent. It exhibits the average weights of two animals which were deprived of both substances for a somewhat longer period than in the earlier experiments (Chart II). In very marked contrast is the result of feeding when either one of the two is present in the diet. The two middle curves of Chart III show the behaviour of rat-pairs when receiving in two strictly comparable experiments either histidine without arginine (upper of these curves) or arginine without histidine (lower curve). There is no loss of weight such as follows when both are absent, but maintenance, and even slight growth. These results were obtained when the amount of arginine or histidine respectively amounted to only 3 % of the total amino-acid mixture. In another experiment (uppermost curve) histidine was added to the extent of rather under 5 % of the whole mixture and appreciable growth followed upon the diet, although arginine was absent. These results receive further attention in a later discussion.

Effects upon Purine Metabolism. We shall now give the protocols of experiments in which the allantoin excretion was determined week by week when arginine and histidine were first removed from, and afterwards restored to, the diet.

It will be seen from colums 4 and 7 of the protocol that in Experiment I the replacement of a bread and milk diet by one containing an amino-acid mixture deprived of the two diamino-acids, led, by the second week of the substitution, to a fall of over 40 % in the allantoin excreted. The excretion rose somewhat in the third week, but during the fourth it was only one half of the amount shown on the normal diet. When, in the sixth week of the experiment, arginine and histidine were restored to the amino-

acids of the diet the excretion of allantoin immediately rose, and after three weeks of feeding on the completed mixture it reached nearly 90 % of its original value.

In Experiment II, done under similar conditions, there was a fall of 47 % during the second week of deficiency feeding, and the results, in general, are very similar to those of Experiment I, save that there was a degree of

Arginine and Histidine absent from Food.

Experiment I.

Week 1914	Mean weight during week: Rat *A*	Rat *B*	Allantoin from two rats per week	Nitrogen per rat per day: Total N	Purine N	Allantoin N	Allantoin N as percentage of total N.	Diet
1 (18. VI.–25. VI.)	124	132	0·458	0·147	0·00065	0·0116	7·8	Bread and Milk
2	116	126	0·296	0·095	0·00044	0·0080	8·4	Amino-acids without arginine or histidine
3	109	116	0·260	0·107	0·0003	0·0065	6·3	,, ,, ,,
4	105	109	0·319	0·116	0·0004	0·0080	6·7	,, ,, ,,
5	102	106	0·228	0·104	0·00023	0·0058	5·6	,, ,, ,,
6	113	114	0·347	0·134	0·00036	0·0090	6·7	Amino-acid + A. and H.
7	121	124	0·347	0·133	0·00034	0·0090	6·7	,, ,, ,,
8	131	131	0·400	0·157	0·00049	0·1010	7·0	,, ,, ,,
9	137	141	0·454	0·199	0·00069	0·1150	5·8	Bread and milk

Experiment II.

	Rat *C*	Rat *D*						
1	146	125	0·575	0·182	0·00063	0·0146	8·0	Bread and milk
2	139	119	0·406	0·105	0·00044	0·0103	9·8	Amino-acids – A. and H.
3	125	112	0·306	0·111	0·00046	0·0077	7·0	,, ,, ,,
4	119	106	0·390	0·142	0·00037	0·0099	7·0	,, ,, ,,
5	120	106	0·310	0·130	0·00039	0·0079	6·1	Amino-acids + A. and H.
6	125	115	0·413	0·119	0·00046	0·0105	8·8	,, ,, ,,
7	131	120	0·400	0·135	0·00053	0·0099	7·3	,, ,, ,,
8	133	127	0·486	0·136	0·00050	0·0123	9·0	,, ,, ,,
9	142	137	0·501	0·167	0·00051	0·0136	8·1	Bread and milk

lag in the effects of restoration. In the first week of giving the completed amino-acid mixture the excretion of allantoin remained nearly at its lowest, but it rose rapidly in the two succeeding weeks.

In the above experiments the food of the first control period was a purine-free, but otherwise normal, dietary (bread and milk). We have found that usually, though not always, a fall from 10 to 15 % in the allantoin seems to follow when the food is changed from bread and milk to a dietary containing even a complete amino-acid mixture. This result does not depend

upon any general failure of nutrition, as it may occur when the weight is going up and the total consumption of nitrogen greater than during the bread and milk period. It is not easy to explain. The bread and milk consumed by two rats during a week would—to judge from determinations made by one of us [Ackroyd 1911]—contain enough allantoin to add 10 to 12 mgs. to the week's excretion. It might possibly add more, but not enough to account for the result mentioned. If such views as those of Marès [1888] are accepted it is possible that because liberated amino-acids instead of intact protein enter the alimentary canal there is less activity of the digestive glands and therefore some lessening of purine metabolism. Possibly the fact that the free acids are absorbed rapidly, rather than gradually as during normal digestion, may affect the efficiency of synthesis in the body. Growth upon the complete amino-acid mixture though satisfactory is seldom of normal velocity.

In any case the circumstance in no way affects the significance of the experimental results. Experiments I and II should be compared with Experiments VI and VII which were done under precisely similar conditions.

In the two experiments which follow a complete amino-acid mixture formed the nitrogen supply of the preliminary, as well as of the final, control period.

It may be further noted that in the first two experiments when arginine and histidine were restored to the diet they were not isolated but were added as recovered together from the silver-barium precipitate of the Kossel and Kutscher process. In the later cases they were added pure, in the form respectively of the nitrate and hydrochloride. Sodium carbonate was simultaneously added in quantity sufficient to correspond with the acid radical of the salt.

In Experiment III in which during the preliminary control period the complete amino-mixture and not bread and milk was given, the urine during the first week of feeding without arginine and histidine was unfortunately lost. That of the second week of the deficiency still contained nearly 85 % of the original output of allantoin. There was apparently therefore a delay in the effect of the deficiency not seen in the first experiments. During the third and fourth weeks however the allantoin fell to 60 % of its original value, and then rose immediately when the arginine and histidine were restored.

In Experiment IV the allantoin was sharply reduced when arginine and histidine were removed from the complete mixture of amino-acids first

given. In the second week of the deficiency it had fallen to one half. It rose somewhat in the two succeeding weeks, but in the fourth week it was only 64 % of its original value. On restoration of the missing amino-acids it quickly rose to the original amount.

Although the time incidence of the minimum varied in the above experiments the removal of arginine and histidine from the food never failed to produce a noteworthy decrease in the allantoin excreted.

Arginine and Histidine absent.

Experiment III.

Week 1915	Mean weight during week		Allantoin from two rats per week	Nitrogen per rat per day				Diet
	Rat *E*	Rat *F*		Total N	Purine N	Allantoin N	Allantoin N as percentage of total N	
1 (11. v.–18. v.)	136	125	0·366	0·121	—	0·0092	7·6	Complete amino-acid mixture
2	134	123	—	—	—	—	—	Arginine and histidine absent
3	127	115	0·312	0·120	—	0·0079	6·6	,, ,, ,,
4	126	114	0·237	0·110	—	0·0060	5·5	,, ,, ,,
5	123	110	0·221	0·112	—	0·0058	5·2	,, ,, ,,
6	137	113	0·356	0·150	—	0·0090	6·0	Arginine and histidine restored
7	149	121	0·356	0·147	—	0·0090	6·1	,, ,, ,,
8	155	128	0·343	0·130	—	0·0087	6·7	,, ,, ,,

Experiment IV.

	Rat *G*	Rat *H*						
1	124	115	0·400	0·118	—	0·0101	8·5	Complete amino-acid mixture
2	129	123	0·406	0·115	0·00051	0·0103	9·0	,, ,, ,,
3	120	118	0·301	0·107	0·00039	0·0076	7·1	Arginine and histidine absent
4	113	111	0·210	0·109	—	0·0053	4·8	,, ,, ,,
5	110	106	0·290	0·101	0·00029	0·0073	7·2	,, ,, ,,
6	106	102	0·256	0·098	0·00023	0·0065	6·6	,, ,, ,,
7	112	109	0·374	0·110	—	0·0095	8·6	Arginine and histidine restored
8	119	115	—	0·114	—	—	—	,, ,, ,,
9	126	119	0·405	0·121	0·00057	0·0102	8·4	,, ,, ,,

Experiments V and V*a* show the effects produced upon the allantoin when of the two diamino-acids only one is absent from the food. In the first arginine was present and histidine absent; in the second arginine was alone wanting. In the control periods the animals received the complete amino-acid mixture. In neither case was the fall at any period greater than 17 %. This is in marked contrast with the 40 to 50 % decrease induced by the absence of both substances. We hold that this interesting result supports the evidence from the body weight curves in suggesting that arginine and histidine are essentially equivalent in metabolism.

To control the experiments already described and to decide so far as possible whether the reduction of allantoin represents the failure of a specific synthesis in the body, and is not an indirect phenomenon depending upon the general failure in nutrition, we have studied the effect of other deficiencies.

The effect of removing tryptophane was first investigated. In Experiments VI and VII the diet of the first control period was bread and milk. This was immediately followed by food containing an amino-acid mixture

Histidine or Arginine removed.

Experiment V.

Week	Mean weight during week JA	KA	Allantoin from two rats per week	Nitrogen per rat per day: Total N	Allantoin N	Allantoin N as percentage of total N	Diet
1	120	122	0·410	0·170	0·0104	6·1	Total amino-acids
2	120	125	0·390	0·171	0·0099	5·8	Arginine present, histidine absent
3	118	127	0·351	0·152	0·0089	5·8	,, ,, ,,
4	115	127	0·337	0·140	0·0086	6·1	,, ,, ,,
5	114	128	0·343	0·134	0·0087	6·5	,, ,, ,,
6	114	125	0·343	0·130	0·0087	6·7	,, ,, ,,

Experiment V*a*.

	JH	KH					
1	135	131	0·448	0·189	0·0106	5·6	Total amino-acids
2	134	130	0·441	0·180	0·0105	5·8	Histidine present, arginine absent
3	137	131	0·400	0·144	0·0099	6·9	,, ,, ,,
4	138	139	0·374	0·137	0·0095	6·9	,, ,, ,,
5	137	138	0·374	0·142	0·0095	6·7	,, ,, ,,
6	134	131	0·356	0·140	0·0090	6·4	,, ,, ,,

deprived of tryptophane. These two experiments are therefore to be compared with Experiments I and II. In neither do we find the effect upon allantoin produced by the absence of arginine and histidine. In Experiment VI it is true a disturbance in the purine metabolism is shown which it is not easy to explain. After two weeks of the deficiency, showing no appreciable effect upon the allantoin, there was in the third week a sudden rise, and, in the following week what seems to be a compensating fall. The average of the two weeks represents an excretion which was nearly normal.

In Experiment VII there was no irregularity of this sort. The replacement of bread and milk by an amino-acid diet without tryptophane was associated with a maximal fall —in the second week—of 19 %. In the third week the excretion was only 9 % below that of the bread and milk period. Restoration of tryptophane to the diet produced no rise.

In Experiments VIII and IX the diet of the preliminary control period was one containing the complete amino-acid mixture. They are therefore comparable with Experiments III and IV. The removal of tryptophane produced no effect upon allantoin at all comparable with that seen when arginine and histidine are absent. In certain weeks when upon the deficiency diet the excretion of the animals was above that of the control period.

Tryptophane absent from food.

Experiment VI.

Week 1914	Mean weight during week		Allantoin from two rats per week	Nitrogen per rat per day			Diet
	Rat *M*	Rat *N*		Total N	Allantoin N	Allantoin N as percentage of total N	
1 (4. XI.–13. XI.)	106	98	0·410	0·170	0·0104	6·1	Bread and milk
2	118	105	—	—	—	—	,,
3	118	107	0·392	0·198	0·0098	4·9	Amino-acids, tryptophane absent
4	111	100	0·410	0·171	0·0104	6·1	,, ,, ,,
5	103	94	0·536	0·150	0·0136	9·0	,, ,, ,,
6	98	90	0·245	0·126	0·0062	4·9	,, ,, ,,
7	109	100	0·326	0·136	0·0083	6·1	Tryptophane restored
8	129	117	0·380	0·154	0·0096	6·2	,, ,,
9	135	122	0·406	0·167	0·0103	6·2	Bread and milk

Experiment VII.

	Rat *O*	Rat *P*					
1	107	107	0·393	0·141	0·0101	7·2	Bread and milk
2	107	107	0·351	0·157	0·0089	5·7	Amino-acids without tryptophane
3	102	102	0·335	0·160	0·0085	5·3	,, ,, ,,
4	91	92	0·357	0·153	0·0095	6·2	,, ,, ,,
5	92	95	—	0·130	—	—	Total amino-acids
6	104	98	0·319	0·123	0·0081	6·5	,, ,,

In Experiment X the periods observed were short. It is of interest however because in it the same rats were fed first without arginine and histidine and then, after an interval with normal feeding, without tryptophane. A marked fall in the allantoin (40 %) occurred under the former conditions, and a much smaller one (10 %) in the latter. In each case the food of the preliminary period was bread and milk.

The difference between the effect of removing tryptophane and that of removing arginine and histidine is throughout the experiments quite unmistakable.

Tryptophane absent.

Experiment VIII.

Week 1915	Mean weight during week: Rat *S*	Mean weight during week: Rat *T*	Allantoin from two rats per week	Nitrogen per rat per day: Total N	Nitrogen per rat per day: Allantoin N	Allantoin N as percentage of total N	Diet
1 (10. II.–15. II.)	141	134	—	—	—	—	Total amino-acids
2	152	141	0·319	0·104	0·0081	7·8	,, ,,
3	146	135	0·319	0·179	0·0081	4·5	Tryptophane absent
4	130	122	0·347	0·160	0·0088	5·5	,, ,,
5	118	114	0·300	0·151	0·0076	5·1	,, ,,
6	109	108	0·304	0·130	0·0077	5·9	,, ,,
7	110	108	0·308	0·102	0·0078	7·6	Tryptophane restored
8	118	113	0·276	0·114	0·0070	6·1	,, ,,

Experiment IX.

	Rat *SS*	Rat *TT*					
1	220	225	—	—	—	—	Total amino-acids
2	215	222	0·347	0·276	0·0088	3·2	,, ,,
3	202	207	0·400	0·277	0·0099	3·6	Tryptophane absent
4	189	193	0·343	0·286	0·0087	3·1	,, ,,
5	176	178	0·300	0·196	0·0075	3·8	,, ,,
6	171	172	0·400	0·266	0·0099	3·7	,, ,,
7	181	179	0·356	0·202	0·0090	4·4	Tryptophane restored
8	199	197	0·420	0·322	0·0107	3·3	,, ,,

Experiment X.

Week 1914	Mean weight during week or period: Rat *AA*	Mean weight during week or period: Rat *BB*	Allantoin from two rats per week	Nitrogen per rat per day: Total N	Nitrogen per rat per day: Purine N	Nitrogen per rat per day: Allantoin N	Diet, etc.
1 (6. III.–13. III.)	210	201	0·400	0·200	0·0010	0·0101	Bread and milk Three days on amino-acids minus arg. and hist.
2 (16. III.–23. III.)	182	188	0·239	0·1910	0·0006	0·0060	ib. (week)
3 (30. III.–5. IV.)	187	189	0·325	0·220	0·0008	0·0082	Total amino-acids Rats for three weeks on bread and milk
Ten day periods							
1	242	262	0·512	0·259	0·0010	0·0130	Bread and milk
2	228	251	0·458	0·229	0·0008	0·0116	Amino-acids minus tryptophane
3	213	242	0·458	0·2300	0·0008	0·0116	,, ,, ,,
4	215	244	0·441	0·1700	0·0005	0·0112	Tryptophane restored
5	259	278	0·473	0·225	0·0012	0·0120	Bread and milk

We have in one experiment studied the effects of quite another type of dietetic deficiency upon the allantoin excretion—the lack, namely, of exogenous growth hormones, or vitamines. As this involves nutritive failure of a marked kind the results offer further control of the arginine and histidine results.

Two rats were fed for eight weeks upon a synthetic diet containing purified caseinogen. During the first four weeks each animal received 2 cc. of fresh milk a day [Hopkins 1912]. In the last four weeks this was withheld, and, as the result of the absence of vitamines, the weight of the animals, which had risen during the first period, then fell rapidly, especially in the last two weeks. We give the results of the experiment in brief form.

	With Vitamine supply				Without Vitamine supply			
Week	1	2	3	4	5	6	7	8
Allantoin from two rats per week	0·263	0·319	0·265	0·285	0·294	0·350	0·340	0·296
Total N from two rats per week	2·47	2·83	2·91	—	3·25	3·38	4·08	3·50

The increase in food consumption after the withdrawal of the small dose of milk has been observed before. It was somewhat disconcerting in the present experiment. Nevertheless the first period was one of growth, and the second one of declining weight. We have further proof therefore that the decrease in allantoin due to the withdrawal of arginine and histidine is not due to nutritive failure alone, for in this experiment there was no fall.

It may be asked here how far the excretion of allantoin in the rat changes with increase or decrease of the total nitrogenous metabolism. All that we know concerning purine metabolism in other animals when the diet is purine-free would lead us to expect that there would be no direct proportionality.

In the protocols of our main experiments will be found a column giving the allantoin-nitrogen as a percentage of the total nitrogen. It will be seen that in general the relation is irregular because of disproportionate and independent variations in the two quantities. It may be noted however that the ratio of allantoin-N to total-N always tended to fall markedly as the result of arginine and histidine deficiency.

We carried out one brief experiment intended to bear specially on the relation of allantoin to total nitrogen. A pair of rats was for one week fed upon a diet of bread and water. Then for one week a large proportion of

caseinogen was supplied, while in a third week the animals returned to bread and water. The results were as follows:

	1st week	2nd week	3rd week
Allantoin	0·408	0·404	0·465
Total Nitrogen ...	2·26	4·27	2·35
Ratio of A.-N. to T.-N.	1 : 16	1 : 30	1 : 14

In this particular experiment the moderate rise in allantoin which occurred was not seen until the animals were again upon the diet containing the lower proportion of protein. Our main experiments were clearly not of a kind to afford evidence upon the point. The distinction between endogenous and exogenous purine excretion is, however, known to be vaguer than was once thought. A large increase of protein in the food has been shown to increase the output of purines, at least in some animals.

The behaviour of the tissues complicates the phenomena. We may perhaps picture the relations in the following way. Actual starvation increases the excretion of endogenous purines because of tissue breakdown. Once equilibrium is re-established on a proper food supply, the normal activities of the tissue determine and limit the amount of purine production through a somewhat wide range of protein intake; until ultimately, when the intake is large enough, the chemical reactions involved are inevitably accelerated by the continued high concentration of new material in the cell. It is clear that if a particular constituent of the protein molecule rather than any other is concerned in purine synthesis, experiments in which the tissues were at first wholly deprived of that constituent are those most likely to demonstrate the fact.

Discussion of Results.

Our observations show that the simultaneous removal of arginine and histidine from its food produces a marked decrease in the excretion of allantoin by the rat. The excretion observed when a normal purine-free diet was given, or that upon a diet containing a full assembly of amino-acids, was reduced by some 40 to 50 % during the period (1 to 4 weeks) when the animals were deprived of these two diamino-acids. The actual minimum of allantoin occurred sometimes early and sometimes later, but throughout the period of the deficiency it was always notably below the normal. On restoration of the missing diamino-acids it returned to or approached its original level. These effects seem to be definite and constant.

Since however deprivation of the two diamino-acids produces at the same time a marked effect upon the general nutrition of the animal, involving at first a rapid loss of weight, it is clear that the relation thus brought to light cannot be at once accepted as a proof that arginine and histidine are direct precursors of the purines of the body. The effect of their removal upon the allantoin excretion might be indirect, and a partial expression of the general nutritional failure. We know of no information in the present literature of the subject which could assist in measuring this possibility.

In actual starvation, to judge from Cathcart's observations upon a fasting man [Cathcart 1907], there is first a brief fall and, later, a rise in the purine excretion. The fall in the case studied by Cathcart lasted for three days only. A period of three or four days in the case of a man is out of all proportion small when compared with one of three or four weeks in the case of a rat. The brief fall at the beginning of starvation depends certainly upon factors different from those responsible for our results.

Moreover a qualitative deficiency in the amino-acid supply cannot be compared with actual starvation. The animals in our experiments were receiving an abundant energy supply throughout the periods observed, in spite of the failure in nutrition, and their nitrogenous metabolism was sometimes above the normal. There was no experimental information which could enable us to judge as to what might be the indirect effect if any of a general nutritive condition of this sort upon the special phenomena of purine metabolism.

By far the best method of controlling our results was—it seemed to us—to compare the effects of the deficiency in arginine and histidine with those of another deficiency in the diet, and, especially, of another amino-acid deficiency. We therefore fed rats upon a diet of which the nitrogenous supply was contained in an amino-acid mixture deprived of tryptophane. In the absence of this constituent the disturbance in the nutritional balance and the loss of weight are even more marked than when arginine and histidine are absent. In these control experiments all other factors in the diet, and all the other experimental conditions were made identical with those of the main experiments. The relative proportions of carbohydrate and fat, for instance, were the same. The only variation was in the nature of the amino-acid or acids removed from the food. Such a comparison should eliminate the influence of many unknown or uncertain factors.

Examination of the protocols given in the last section will show that the removal of tryptophane produced no fall in the excretion of allantoin

at all comparable with that seen when arginine and histidine are absent. The excretion was somewhat irregular; at times there was a small fall during part of the deficiency period, at other times there was some increase; on one occasion there was a considerable rise during the third week followed by a compensatory fall in the fourth. Taking the results as a whole however the difference between the effects of removing tryptophane and those which follow the removal of arginine and histidine is sharp and unmistakable. In the latter case the decrease in allantoin is constant and of marked degree.

It happens that in the case of the tryptophane deficiency the mean weekly excretion of the animals (17 weekly estimations) was identical with that of animals upon the complete amino-acid mixture (25 estimations) namely 0·135 g. allantoin per rat per week. This may be a coincidence but it is good evidence that there was no decrease of any importance.

In no case, it should be noticed, did we extend our observations concerning the allantoin excretion beyond the fourth week of feeding upon the deficient amino-acid mixture. At this stage and sometimes sooner we restored the missing constituent to the diet in order to control the results. We do not know what may happen to the allantoin at later periods. For the specific purpose of the research it seemed better to compare the effect of the different diets upon purine metabolism when the nutritional failure was as yet not too marked. Premortal changes might introduce complications and obscure the result.

A dietetic deficiency of quite another kind, the absence namely of growth hormones or vitamines, produced, in the one experiment carried out to test the matter, no fall in the excretion of allantoin, although the animals were losing weight as fast as those fed without the diamino-acids.

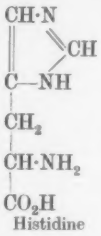
All our results therefore point to the probability that there is a specific metabolic relation between arginine and histidine and the purines of the body.

It was explained in the introductory paragraphs of this paper that we began our experiments in the belief that the molecules of arginine and histidine might prove to be used conjointly in metabolism as precursors of the purine ring. Our observations upon body weight, and, no less, others upon allantoin excretion seem to show clearly however that either one of these two diamino-acids can with a considerable degree of efficiency subserve the functions of the other. When both are removed from the diet the nutritive failure and the fall in allantoin excretion are unmistakable. When

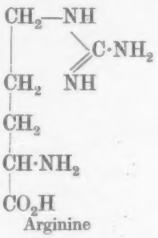
either alone is restored in sufficient amount there is maintenance and even growth, while no marked fall in allantoin is observed.

On our own view this is because each of these protein derivatives is, in metabolism, capable of being converted into the other, and is so converted when the supply of both is not harmoniously adjusted for the needs of the animal.

The chemical relations which justify this view are familiar, and become clear when the structural formulae are properly compared.



Histidine



Arginine

If arginine and histidine are, in the sense just discussed, to a large extent, equivalent in metabolism our original view that the molecules of both, rather than the molecule of histidine alone, are concerned in the synthesis of the purine ring loses some of its point. That the presence of both in the food yields the optimum conditions for purine synthesis seems nevertheless to be probable.

Summary.

(1) When arginine and histidine are together removed from the diet of rats which have been previously growing on a complete amino-acid mixture there is a rapid loss of body weight. This is promptly succeeded by renewed growth when the missing diamino-acids are restored to the diet.

(2) When arginine alone, or histidine alone, is restored to the food there is no loss of weight and there may even be growth. Nutritional equilibrium is possible in the absence of one of these related protein constituents though not in the absence of both. It is suggested that this is because each one of them can, in metabolism, be converted into the other.

(3) When arginine and histidine are both removed from the food the amount of allantoin in the urine is much decreased. When they are replaced the excretion returns to the normal. The decrease is very much less when either one of these diamino-acids is present alone.

(4) No decrease of allantoin excretion occurs when tryptophane is removed from the food, though nutritional failure is even greater than when arginine and histidine are withheld. No decrease was observed when animals were losing weight as the result of the absence of a vitamine supply.

(5) It is suggested therefore that arginine and histidine play a special part in purine metabolism, probably constituting the raw material (or the most readily available raw material) for the synthesis of the purine ring in the animal body.

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